

# **EUP No 86414-EUP-1 Vol 1 of 2**

March 16, 2012

Jennifer Urbanski, Ph.D.  
US Environmental Protection Agency  
Insecticide-Rodenticide Branch, S7221 Registration Division (7505P)  
1200 Pennsylvania Ave. NW  
Washington, DC

**Re: Transmittal of Sampling and Analysis Plan to Support EPA Issuance of an Experimental Use Permit for Field Testing of Imidacloprid to Control Burrowing Shrimp; and Responses to EPA Comments Raised in Previous Correspondence.**

Dear Dr. Urbanski:

Attached please find a copy of the Sampling and Analysis Plan (SAP) we have developed to describe our proposed 2012 field and laboratory testing of imidacloprid in Willapa Bay, Washington. Imidacloprid is being proposed for use in commercial shellfish beds by the Willapa Grays Harbor Oyster Growers Association (WGHOGA) to control two species of burrowing shrimp that, if left untreated, cause significant oyster mortality in such beds. WGHOGA is proposing to use imidacloprid, a selective, neonicotinoid pesticide with low toxicity to vertebrates, instead of carbaryl, which the growers have used to control burrowing shrimp since 1963.

The SAP was designed to meet format and content guidelines of the Washington State Department of Ecology (Ecology). Ecology has provided multiple rounds of written and verbal informal comments on earlier drafts of the SAP. After receiving this input from Ecology, we submitted the attached version of the SAP to Ecology for formal review and approval on March 15. We very much appreciate EPA's willingness to wait a few weeks before commencing review to allow Ecology to provide informal feedback. This coordination has facilitated EPA's and Ecology's current simultaneous review of our final proposal for 2012 field efforts.

The attached SAP describes the locations and application methods associated with imidacloprid trials in 2012, including extensive information on the sampling of water, sediments, invertebrates, and eelgrass that will be conducted to help assess the ecological effects of imidacloprid use. The SAP also discusses how samples will be processed, shipped, and analyzed by a state certified analytical laboratory. Finally, the SAP outlines how the collected data will be checked for quality, and then evaluated to determine ecological effects, including the Sediment Impact Zone (SIZ) that





must be delineated under state regulations. We look forward to EPA's comments on the attached SAP.

Previously, WGHOGA's representatives submitted various research plans containing much of the same information to EPA, which responded with two sets of written comments:

- **Review of sampling analysis protocol for the use of imidacloprid on oyster beds under an experimental use permit. DP Barcode: 391941, 391695; PC Code: 129099; Date: 08/11/11**
- **Experimental Use Permit for Imidacloprid Products Protector 2F and Protector 0.5G for Control of Burrowing Shrimp on Oyster Beds in Washington State. DP Barcode: D384152; PC Code: 129099**

We appreciate the time and effort that EPA has invested in order to develop and provide these comments. EPA's comments are focused on optimizing the sampling design and methods in keeping with guidance documents and past practice. We understand both the scientific value and the role of procedural precedence in many of EPA's suggestions and questions regarding the sampling analysis protocol and EUP application we had previously provided. We have attached responses to the comments and concerns raised by EPA in these two submissions. We have also incorporated EPA's input into the attached version of the SAP. We hope these responses and the revised SAP will satisfactorily address EPA's concerns.

Recall that with submission of the SAP that Ecology will commence a formal review of that document. We have conducted three sets of meetings with Ecology to work through questions and issues with the SAP in advance of formal submittal in a joint effort to reduce the need for future changes. Hence, we are hopeful that EPA's review will not be complicated by any future Ecology-required substantive changes to the plan. However, we will inform EPA immediately if substantive changes are required.

It is evident that EPA and its staff have spent considerable time and effort to develop input designed to improve our work and its utility for agency and public review. Thank you again for that time and effort.

Without a 2012 Experimental Use Permit, WGHOGA will be unable to proceed with implementation of the SAP, which is required in order to apply for a NPDES permit from Ecology. WGHOGA is scheduled to phase out carbaryl at the end of 2012 pursuant to a private settlement agreement. The lack of an authorized viable alternative to carbaryl puts the entire Willapa Bay and Grays Harbor oyster industry at risk. This represents the bulk of the West Coast oyster industry.



WGHOGA  
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Accordingly, we are quite motivated to assist EPA in its review of our EUP application. So please do not hesitate to contact us if we may be of assistance. In particular, we reiterate our willingness to meet or have a conference call involving our scientists with EPA staff to help explain our proposed 2012 SAP, and to discuss any EPA questions or concerns.

Sincerely,

**HART CROWSER, INC.**

**JEFFREY C. BARRETT, PH.D.**  
Regional Manager, Hart Crowser, Inc.

**WASHINGTON STATE UNIVERSITY**

**KIM PATTEN, PH.D.**  
Extension Professor

**Attachments:**

Appendix A - Sampling and Analysis Plan

Appendix B - Responses to Previous EPA Correspondence

**CC:**

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## **Field trials of imidacloprid against burrowing shrimp, 2011**

Dr. Kim Patten, Washington State University Long Beach Research and Extension Unit

### **Introduction**

Research conducted from 2006 to 2010 has suggested that imidacloprid may be a good alternative to carbaryl for controlling burrowing shrimp. The research effort has focused on refining efficacy of imidacloprid using different timings, rates, formulations, sediment types and conditions and application methods. On January 11, 2011, the EPA granted Federal Experimental Use Permits 86414-EUP-1 and 86414-EUP-2 to Dr. Kim Patten to apply liquid and granular imidacloprid in Willapa Bay. 30 lbs a.i. of Mallet, at a maximum rate of 1 lb a.i. per acre, and 90 lbs a.i. of Nuprid, at a maximum rate of 2 lbs a.i. per acre, were authorized for application. In the case that Nuprid or Mallet were to be applied at lower than maximum rates, the total acreage of beds treated with imidacloprid was not to exceed 90.

### **Objectives**

- Assess and compare the efficacy of liquid and granular formulations of imidacloprid against burrowing shrimp at a commercial scale on plots of differing vegetation density and substrate.
- Compare methods of applying granular imidacloprid.
- Assess the impact of imidacloprid at the commercial scale on non-target fish.
- Assess the impact of imidacloprid to the benthic infauna.
- Measure the associated concentrations of imidacloprid in the water column, pore-water, and in sediments.
- Further validate the precision and accuracy of an ELISA analytical technique compared to the standard HPLC technique.
- Survey plots following application for impact on macrobenthic organisms, especially crabs and fish.

### **Methods**

In 2011, a total of 51.38 acres, all large plots (>0.1 ac) on intertidal commercial shellfish beds not currently farmed, were treated with imidacloprid (Figures A1 to A5, Table A1). There were 18 separate experiments. These comprised a total of 29.54 ac and 21.84 ac of Nuprid 2F and Mallet 0.5G, respectively. Detailed information about treatments, treatment sites, and applications are listed in Tables A1 to A3. Locations of treatment sites are provided in Figures A1 to A5. Efficacy (14 days after treatment) and impacts to Dungeness crab (24 hours after treatment) were measured on all sites. Efficacy was assessed by counting shrimp burrows before and after treatment, and/or relative to an untreated control site. Whole bed density of epibenthic megafauna (Dungeness crab and fish) were assessed by making multiple closely spaced transects over the beds counting all affected megafauna species on and within 150 feet of the site. Affected species were those exhibiting any signs of tetany, or were dead by any cause, directly or indirectly related to the treatment (e.g. bird predation of tetany crab).

At four treatment sites, along with comparable control sites, imidacloprid was measured in eelgrass vegetation, water and sediment pore water before and after treatment. These sites were two 10 acre treatment beds near Palix River (Nuprid 2F at 0.5 lb a.i./ac and Mallet 0.5 G at 0.5 lb

a.i./ac), and two five acre treatment beds near the Cedar River site (Nuprid 2F at 0.5 lb a.i./ac and Mallet 0.5 G at 0.5 lb a.i./ac). Samples were collected at fixed sample points to maximize on-bed detection and off-bed movement (Figures A6, A7 and A8). Off-bed sample points were chosen by observing incoming and outgoing tide patterns and picking locations that funneled the highest volume of water over the treated area. Samples were collected at predetermined time intervals before and after applications (Tables 5 to 8).

Ebb flow tidal water was sampled immediately after treatment for Mallet application, 0 hours after treatment (0 HAT). Samples were collected at the bed boundary, and at distances from 30 to 240 m in the direction of tidal ebb flow. Because water depth will vary across each station at the time of sampling, ebb water was collected from the middle of the water column until the jar was filled. For both Mallet and Nuprid sites, the first incoming tidal water after treatment was sampled at the bed center and near edge and extended out up to 240 m from the treatment area in the direction of water moving off the bed.

Additional water samples were collected at the center of beds at 6, 24 and 54 HAT for Palix River sites and 54 and 102 HAT at the Cedar River sites. Water samples were collected in 1 L amber glass jars. Incoming tidal water depth at sampling at Palix River was when the incoming depth reached ~ 15 cm. Cedar River sites were sampled when the incoming tidal water just flushed over the site (<10 cm). Samples were collected using a "clean hands/dirty hands" protocol and immediately placed on ice after collection, and shipped on ice overnight to Pacific Agricultural Laboratory under chain of custody, where they were stored at 1-4 C and analyzed within the EPA-recommended 7-day holding time. Imidacloprid analysis in water was analyzed using the EPA 8321B (HPLC-MS QQQ) method to a reporting limit of 1.6 µg/l. Quality assurance was by analysis of a method matrix blank and two matrix spike samples with expected percent recovery of 40–120%.

Eelgrass (*Zostera marina*) was sampled from two on-bed sampling points in the middle of the bed for both the Palix River and Cedar River sites. Three off-bed sampling points, 30, 60, and 120 m from the edge of the plot in the main direction of water moving off-site, were added for the Cedar River sites. Samples were collected at 0, 24, 96 and 168 HAT at Palix River and 96 and 336 HAT for Cedar River. To assure the imidacloprid in the eelgrass samples was not from residual water or sediment on the vegetation, 1-liter samples were collected in a 4-liter Zip-loc® bag using "clean hands-dirty hand" protocol. Samples were placed on ice in a cooler and moved off-site to a clean location and then triple rinsed with clean bay water to remove any sediment. Samples were placed in 1 l Nalgene containers, in a dark-colored cooler on ice and shipped overnight on ice to Pacific Agricultural Laboratory, under chain of custody, where they were analyzed for imidacloprid. Imidacloprid analysis for eelgrass water was done by FDA PAM I 302 (HPLC-MS) method to a reporting limit of 0.010 mg/l, with quality assurance by analysis of a method matrix blank and two matrix spike samples with expected percent recovery of 40–120%.

Sediment samples were also collected for sediment pore water and epibenthic and benthic invertebrates. Samples for pore water were frozen to be homogenized, extracted, and analyzed at a later date. Samples for invertebrates were collected and immediately sieved through 0.5 mm mesh using salt water, then stored in a 10% buffered formalin solution and stained with rose



bengal for 1-2 weeks, then re-sieved through 250 µm mesh to remove excess detritus and stored in 70% isopropyl alcohol. These samples will be analyzed at a later date.

### **Results**

Efficacy across all sites and treatments ranged from 42 to 96% burrow reduction (Table A4). Efficacy was highest on sandy sites with no vegetation and lowest on silty sites and vegetated sites. There were no affected fish found on any of the sites following any treatment (data not shown). The number of affected Dungeness crab per site varied from 0 to 19. At sites that were large enough in size (>4 acres) to make valid inferences about potential ecological impact to crab, the number of affected crab per acre ranged from 0.87 to 3.8.

Water sample concentrations for imidacloprid varied by site, location and time. Ebb tidal water from Mallet treatments (0 HAT) were low, 0 to 6 ppb (Tables A5 and A8). Concentrations during the first incoming tide for Mallet ranged from 0 to 68 ppb. All on-bed concentrations of imidacloprid on Mallet sites in subsequent tides were 0 ppb. Concentrations during the first incoming tide for Nuprid treatments ranged from 0 to 1400 ppb (Tables A6 and A7). All on-bed concentrations of imidacloprid on Nuprid in subsequent tides were 0 ppb. Eelgrass vegetation sampled for imidacloprid was 0 ppb for all sites and times of sampling, except for the center of Nuprid bed after 24 hours (24 ppb) (Tables A5 to A8).

### **Discussion**

Mallet and Nuprid at the 0.5 lb ai/ac rate provided adequate efficacy for commercial use to control burrowing shrimp on sandy un-vegetated sediment. However, on silty sites or vegetated sites, Mallet and Nuprid at 0.5 lb ai/ac rate did not always provide enough control to bring shrimp population below the economic threshold level (<2.5 burrows/0.25 m<sup>2</sup>). Additional research will be needed to determine how to enhance efficacy under these conditions.

Treatments appeared to have no significant effect on megafauna. Visual assessment of the treatment sites following applications found no affected fish and only modest numbers of affected Dungeness crab. These results on crab were similar to those observed in previous studies, and were not at a significant enough level to have ecological or commercial consequences.

Concentration on imidacloprid in the water column just above treated sites went to zero within 24 hours after application. Off-site movement of imidacloprid in the water column was noted in both the ebb water during treatment and incoming tidal water after treatment. Imidacloprid concentrations decreased rapidly with distance from the treated site. There was high variability in on and off-bed concentration of imidacloprid in the water column between different sites and locations on and off-bed within a site. These differences reflect imidacloprid's water solubility and the variation in tidal water movement patterns within and between sites.

Imidacloprid does not appear to concentrate in on-site or off-site eelgrass vegetation. It was only detected in one sample (Nuprid on-site 24 HAT).

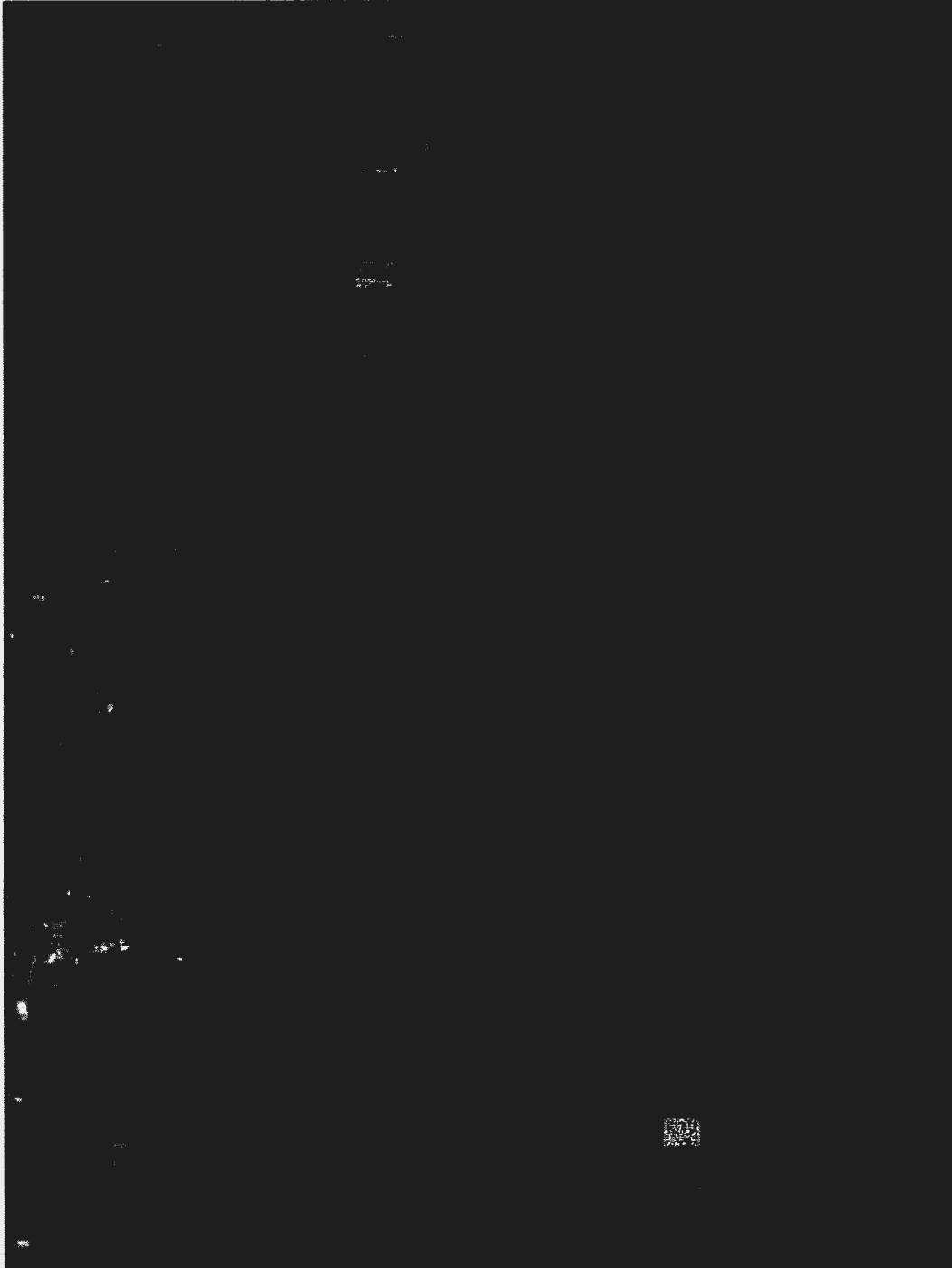
Further discussions of the results are pending analysis of sediment samples for sediment pore water and epibenthic and benthic invertebrates.



Figure A1. Experimental treatment locations.



**Figure A2. Treatment locations in the Leadbetter area. Numbers = treatment area in acres.**



**Figure A3. Treatment locations in Nahcotta. Numbers = treatment area in acres.**





**Figure A4. Treatment locations in Cedar River area. Numbers = treatment area in acres.**



**Figure A5. Treatment locations in Bay Center. Numbers = treatment area in acres.**

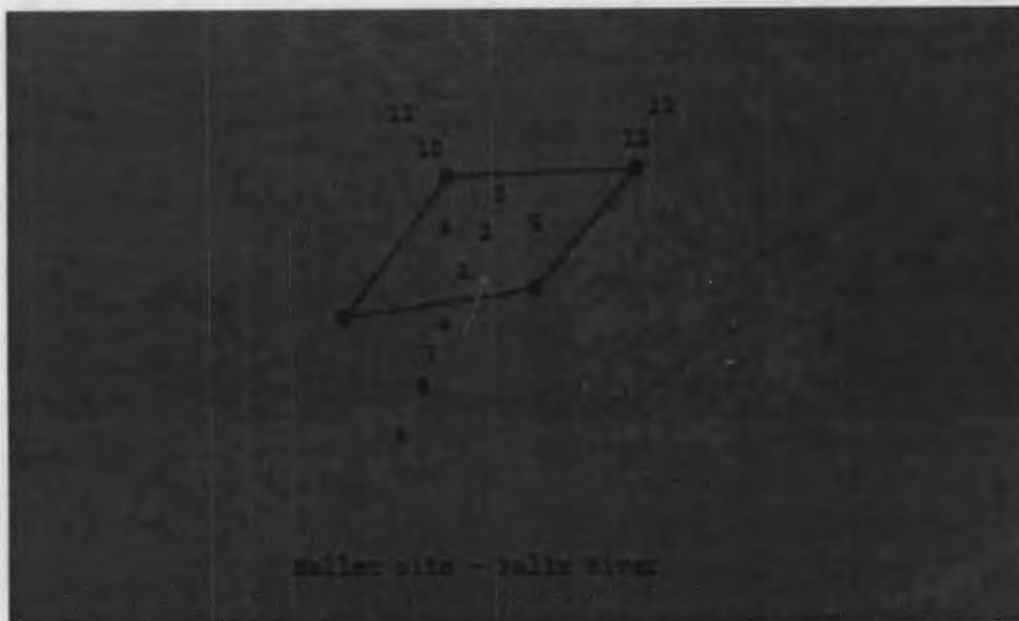


Figure A6. Water and eelgrass sample locations for imidacloprid in 2011. Numbers represent approximate location of samples. Arrow represents direction of incoming tide.

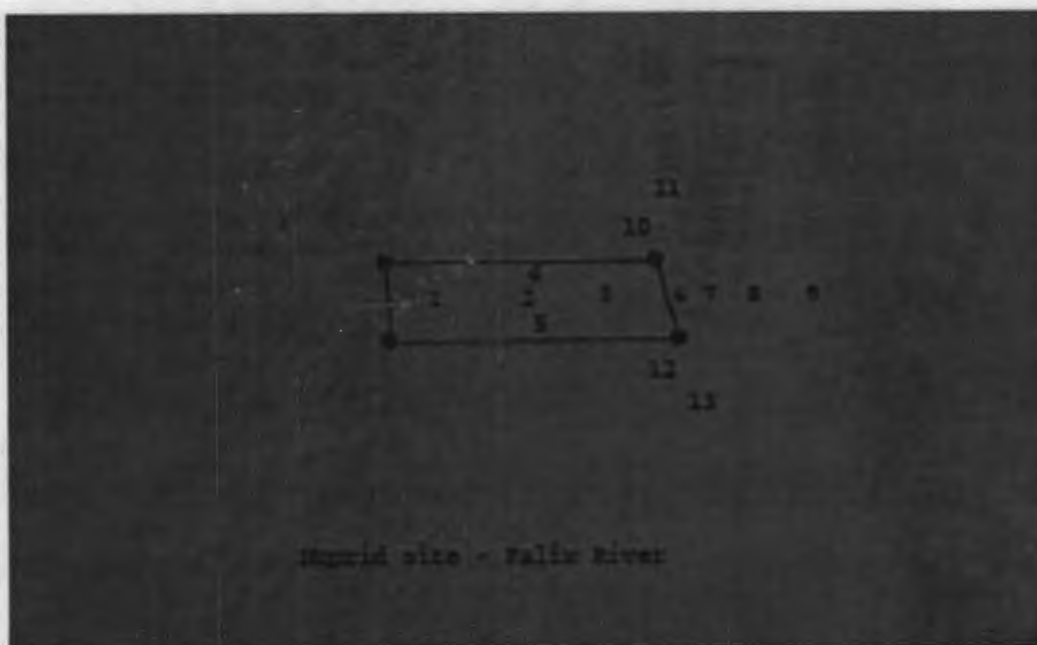


Figure A7. Water and eelgrass sample locations for imidacloprid in 2011. Numbers represent approximate location of samples. Arrow represents direction of incoming tide.



Figure A8. Water and eelgrass sample locations for imidacloprid analysis in 2011. Numbers represent approximate location of samples. Arrows indicate direction of flow of incoming tide.



**Table A1. Characteristics for sites of large plot (>0.1 ac) experimental imidacloprid treatment, 2011**

Ex- peri- ment #	Material	Rate (lb ai/ac)	Bed	Growing area	Acres per plot	# Plots	Acres treated	Treat ment Date	App. Method	Latitude	Longi- tude	Bed Type <sup>a</sup>	Data collected <sup>b</sup>
1	Mallet	0.5	TL 059	Nahcotta N	1	1	1	5/17/11	ATV	46.521	-124.02	Sn Zj	E, C
2	Nuprid	0.5	TL 059	Nahcotta N	1	1	1	5/18/11	ATV	46.521	-124.02	Sn Zj	E, C
3	Mallet	0.5	OB 163E	Nahcotta N	1.7	1	1.7	5/17/11	ATV	46.518	-124.02	Sn Zj	E, C
4	Nuprid	0.5	OB 163E	Nahcotta N	1.7	1	1.7	5/18/11	ATV	46.518	-124.02	Sn Zj	E, C
5	Mallet	0.5	OB 163E	Nahcotta N	0.3	1	0.3	5/17/11	Hand	46.518	-124.02	Sn Zj	E, C
6	Nuprid	0.5	OB 163E	Nahcotta N	0.3	1	0.3	5/18/11	Hand	46.518	-124.02	Sn Zj	E, C
7	Mallet	0.5	OB 163E	Nahcotta N	0.5	1	0.5	6/3/11	Boat	46.515	-124.018	Sn Zj	E, C
8	Mallet & Nuprid	0.5	OB 163E	Nahcotta N	0.06	4	0.24	9/14/11	Hand	46.515	-124.018	Sn Zj	E, C
9	Mallet & Nuprid	0.5	TL 194	Nahcotta S	0.06	2	0.12	5/20/11	Hand	46.494	-124.029	Si	E, C
10	Mallet	0.5	TL 194	Nahcotta S	0.5	1	0.5	6/3/11	Boat	46.494	-124.029	Si	E, C
11	Mallet & Nuprid	0.5	OB E155	Leadbetter	0.3	2	0.6	5/20/11	Hand	46.618	-124.047	Si	E
12	Nuprid	0.5	OB E148	Leadbetter	10.3	1	10.3	7/3/11	Aerial	46.619	-124.036	S	E,C
13	Mallet	0.5	OB B145	Bay Center	2.2	1	2.2	6/2/11	Hand	46.625	-123.969	S	E
14	Mallet	0.5	22	Bay Center	10.2	1	10.2	7/15/11	Aerial	46.636	-123.97	S	E, I, C
15	Nuprid	0.5	OB B290	Bay Center	10.2	1	10.2	7/15/11	ATV	46.621	-123.969	S	E, I, C
16	Mallet	0.5	OB A043	Tokeland	1.4	1	1.4	6/6/11	Boat	46.722	-123.96	Si	E
17	Mallet	0.5	OB A101	Tokeland	4.2	1	4.2	8/31/11	Boat	46.721	-123.959	Si	E, I, C
18	Nuprid	0.50	OB A033	Tokeland	5	1	5	8/31/11	Hand	46.721	-123.954	Si Zm	E, I, C

<sup>a</sup> Sn= sandy, Si=silty, Zj Zostera japonica, ZM Zostera marina, \* Efficacy-E, Imidacloprid-I, Crab- C

<sup>b</sup> E= Efficacy, C= crab on & off bed affected by treatment, I= imidacloprid concentration in water, eelgrass and sediment

**Table A2. Treatment application tidal and weather conditions**

Experi- ment #	Form- ulation	Acres treated	Treatment date	Tidal conditions @ application					Weather conditions @ application					
				Time of application	Water level on bed during application	Low tide/time	Tidal elevation of site	Time of inundation	Water temp (f)	Sediment temp @ 8" (f)	Air tem p (f)	% overcast	Net radiation* (W/m2)	wind
1	Mallet	1	5/17/2011	7:15	0"	-2.7 @ 8:13	+1.1	10:30	59	56	53	0	880	0
2	Nuprid	1	5/18/2011	8:00	0"	-2.7 @ 8:13	+1.0	10:30	59	56	62	0	880	0
3	Mallet	1.7	5/17/2011	7:00	0"	-2.7 @ 8:13	+1.2	10:30	59	56	53	0	880	0
4	Nuprid	1.7	5/18/2011	7:40	0"	-2.7 @ 8:13	+1.2	10:30	59	56	53	0	880	0
5	Mallet	0.6	5/17/2011	7:00	0"	-2.7 @ 8:13	+0.5	10:30	59	56	53	0	880	0
6	Nuprid	0.6	5/18/2011	7:40	0"	-2.9 @ 9:00	+0.5	10:30	59	56	53	0	880	0
7	Mallet	0.5	6/3/2011	6:45 to 7:05	12" to 24"	-2.0 @ 9:30	+0.2	12:30	59	59	53	0	990	5
8	Mallet & Nuprid	0.24	9/14/2011	5:00 to 5:30	0 to 6"	-1.5 @ 9:09	+1.2	11:20	62	62	68	0	990	0
9	Mallet	0.5	5/19/2011	6:00 to 6:30	24" to 36"	-2.0 @ 9:30	+0.9	1:00	59	59	53	0	990	5
10	Mallet & Nuprid	0.12	6/3/2011	7:35 to 8:15	0" to 6"	-2.6 @ 9:50	+1.2	10:00	58	58	51	0	876	3-5 mph NW
11	Mallet & Nuprid	0.6	5/20/2011	7:45 to 8:30	0" to 6"	-1.9 @ 10:35	+1.7	12:00	58	58	58	51	822	3-5 mph NW
12	Nuprid	10.3	7/3/2011	7:00	0"	-2.3 @ 9:45	+1.4	1:15	61		57	100%	280	3
13	Mallet	2.2	6/2/2011	6:30 - 7:30	2" to 4"	-1.9 @ 08:53	+0.4	10:45	55		53	100% w/ light rain	340	10 mph SW
14	Mallet	10.2	7/15/2011	5:20 - 6:10	2" to 6"	-2.5 @ 08:20	+0.4	11:30	63		60	100%	200	0
15	Nuprid	10.2	7/15/2011	7:50 - 9:00	0" to 2"	-2.5 @ 08:20	+0.7	11:45	63		71	65 to 100%	200	2-6 mph SW
16	Mallet	1.4	6/6/2011	7:50 to 8:30	18" to 30"	-1.3 @ 11:30	+0.3	12:15	61		55	100%	370	1 mph NW
17	Mallet	4.2	8/30/2011	4:30 - 6:10	12" to 36"	-0.9 @ 8:31	0.0	9:30 to 10:00	60	65	60	dark	650	0
18	Nuprid	5	8/30/2011	7:30 - 8:50	0" to 6"	-0.9 @ 8:31	0.0	9:30 to 10:00	60	65	60	40 - 80%	650	0

\* Peak net radiation measured during application window, prior to tidal submergence. To convert to ~ uv radiation multiply by 0.052.

**Table A3. Additional site information and application notes.**

Experiment #	Formulation	Acres treated	Treatment date	Other site information			Application notes/ issues
				Bed drainage conditions	Prior site treatments	% coverage by eelgrass	
1	Mallet	1	5/17/2011	good, dry at application	none	10 to 20% but thin canopy coverage @ time of application	moderate uniformity
2	Nuprid	1	5/18/2011	good, dry at application	none	10 to 20% but thin canopy coverage @ time of application	good uniformity
3	Mallet	1.7	5/17/2011	good, dry at application	none	10 to 20% but thin canopy coverage @ time of application	moderate uniformity, with alternating too heavy and too light, west end mostly
4	Nuprid	1.7	5/18/2011	good, dry at application	none	10 to 20% but thin canopy coverage @ time of application	good uniformity
5	Mallet	0.6	5/17/2011	good, dry at application	none	10 to 20% but thin canopy coverage @ time of application	good uniformity
6	Nuprid	0.6	5/18/2011	good, dry at application	none	10 to 20% but thin canopy coverage @ time of application	good uniformity
7	Mallet	0.5	6/3/2011	well drained, water off site w/in 1 hr post treatment	site never treated	0 to 25%	rate and application- good
8	Mallet & Nuprid	0.24	9/14/2011	well drained, water off site w/in 1 hr post treatment	none	0 to 20%	good uniformity, crushing of plot
9	Mallet	0.5	5/19/2011	well drained, water off site w/in 1 hr post treatment	none	0	good uniformity
10	Mallet & Nuprid	0.12	6/3/2011	well drained, dry for Nuprid app.; wet ~ 2" to 4" water for Mallet app.	none	0	good uniformity
11	Mallet & Nuprid	0.6	5/20/2011	well drained, dry at application of Nuprid	none	0	good uniformity

12	Nuprid	10.3	7/3/2011	well drained	site never been treated	0	a few skips in boom pattern
13	Mallet	2.2	6/2/2011	well drained, except for shallow channel W end	site never been treated	0	rate and application - good
14	Mallet	10.2	7/15/2011	well drained, except for shallow channel S end	site never treated, but some areas outside of plots were treated in several yrs. prior	<1%	rate and application - good, but first pass on W edge used to calibrate was heavy
15	Nuprid	10.2	7/15/2011	well drained, except for shallow channel W end that never went dry	site never been treated	0 W half, 10 to 20% E half	rate and application - good
16	Mallet	1.4	6/6/2011	well drained, water off site w/in 1 hr post treatment	site never been treated	<1%	rate and application - good
17	Mallet	4.2	8/30/2011	well drained,	NW section of plot previously treated, excluded from data collection, but treated	<2%	poor uniformity and 1/2x rate on E half of plot
18	Nuprid	5	8/30/2011	SW section remained wet and draining	Previously treated >5 yr ago	5 to 35%	rate and application - good



**Table A4. Treatment efficacy and nontarget impact on Dungeness crab**

Experiment #	Material	Bed Type <sup>a</sup>	App. Method	Acres treated	treatment date	Pre-treatment density <sup>b</sup> (#/0.25m <sup>2</sup> )	Post treatment density (#/0.25m <sup>2</sup> )	% reduction (control)	Total affected Dungeness crab per site 24 HAT <sup>c</sup>	# affected crab/ac <sup>c</sup>
1	Mallet	Sn Zj	ATV	1	5/17/11	4.9	1.4	70	4	4.00
2	Nuprid	Sn Zj	ATV	1	5/18/11	6.9	1.5	78	10	10.00
3	Mallet	Sn Zj	ATV	1.7	5/17/11	5.7	3.3	50	5	2.94
4	Nuprid	Sn Zj	ATV	1.7	5/18/11	7	1.6	74	8	4.71
5	Nuprid	Sn Zj	Hand	0.5	5/18/11	4.3	0.8	81	4	8.00
6	Mallet	Sn Zj	Hand	0.3	5/17/11	7.8	2.2	70	1	3.33
7	Mallet	Sn Zj	Boat	0.3	6/3/11	6.1	2.5	60	2	6.67
8	Mallet	Sn Zj	Hand	0.12	9/14/11	4.5	1.1	75	3.5	29.17
8	Nuprid	Sn Zj	Hand	0.12	9/14/11	4.5	2.6	42	3.5	29.17
9	Mallet	Si	Hand	0.12	5/20/11	8.4	1.8	78	3	25.00
9	Nuprid	Si	Hand	0.12	5/20/11	8.4	1.2	85	0	0.00
10	Nuprid	Si	Boat	0.3	6/3/11	8.4	1.12	86	1	3.33
11	Mallet	Si	Hand	0.3	5/20/11	9.6	3.1	67	1	3.33
11	Nuprid	Si	Hand	0.3	5/20/11	9.6	1	90	1	3.33
12	Nuprid	S	Aerial	10.3	7/3/11	11.1	0.5	96	9	0.87
13	Mallet	S	Hand	2.2	6/2/11	5.2	0.9	82	1	0.45
14	Mallet	S	Aerial	10.2	7/15/11	5.4	0.57	89	5	0.49
15	Nuprid	S	ATV	10.2	7/15/11	5.2	1.2	77	5	0.49
16	Mallet	Si	Boat	1.4	6/6/11	6.4	2.5	61	na	na
17	Mallet	Si	Boat	4.2	8/30/11	6.7	3.5	48	5	1.19
18	Nuprid	Si Zm	Hand	5	8/30/11	4.9	1.9	61	19	3.80

<sup>a</sup> Sn= sandy, Si=silty, Zj *Zostera japonica*, ZM *Zostera marina*, \* Efficacy-E, Imidacloprid-I, Crab- C

<sup>b</sup> Pretreatment counts were not always available, in which case counts from adjacent control sites were used to obtain efficacy (% control).

<sup>c</sup> Affected crab were any crab present across the entire treated area or within 100' around the entire plot that were exhibiting sign of tetany or were dead. Data were collected 24 hours after treatment.

**Table A5. Imidacloprid concentration in water and eelgrass on Palix Bed # 22(10.2 ac) treated with 0.5 lb ai/ac of Mallet on July 15, 2011 by aerial application in 3 to 18 inches of water.**

Treatment	Sample type	Sample location (on/off bed)	Sample location details & location - #	imidacloprid (ppb)				
				Time of sample				
				0 HAT	2 HAT	6 HAT	24 HAT	54 Hat
Control	water				0	0	0	0
Treated	water	on bed	low end -1		56			
Treated	water	on bed	center - 2		57	0	0	0
Treated	water	on bed	high end - 3		82			
Treated	water	on bed	center W - 4		27			
Treated	water	on bed	center E - 5		39			
Treated	water	off bed	30 m low end center - 6	6				
Treated	water	off bed	60 m low end center -7	0.12				
Treated	water	off bed	120 m low end center -8	0				
Treated	water	off bed	240 m low end center -9	0				
Treated	water	off bed	30 m high end NW -10		60			
Treated	water	off bed	60 m high end NW -11		68			
Treated	water	off bed	30 m high end E -12		0			
Treated	water	off bed	60 m high end E -13		0			
				pre	inundation	24 hr	96 hr	168 hr
Control	eelgrass			0	0	0	0	0
Control	eelgrass			0	0	0	0	0
Treated	eelgrass	on bed	center -2	0	0	0	0	0
Treated	eelgrass	on bed	center - 2	0	0	0	0	0

**Table A6. Imidacloprid concentration in water and eelgrass on Palix River Bed # 22(10.2 ac) treated with 0.5 lb ai/ac of Nuprid on July 15, 2011 by ATV broadcast application in 0 to 6 inches of water.**

Treatment	Sample type	Sample location (on/off bed)	Sample location details & location - #	imidacloprid (ppb)				
				Time of sample				
				0 HAT	2 HAT	6 HAT	24 HAT	54 HAT
Control	water				0	0	0	0
Treated	water	on bed	low end - 1		19			
Treated	water	on bed	center - 2		8	0.15	0	0
Treated	water	on bed	high end - 3		9.8			
Treated	water	on bed	center N - 4		4			
Treated	water	on bed	center S - 5		15			
Treated	water	off bed	30 m hi end center -6		7.7			
Treated	water	off bed	60 m hi end center - 7		7.1			
Treated	water	off bed	120 m hi end center - 8		8.8			
Treated	water	off bed	240 m hi end center- 9		1.6			
Treated	water	off bed	30 m hi end N -10		8.2			
Treated	water	off bed	60 m hi end N -11		6.5			
Treated	water	off bed	30 m hi end S - 12		89			
Treated	water	off bed	60 m hi end S - 13		83			
				pre	24 hr	96 hr	168 hr	
Control	eelgrass			0	0	0	0	
Control	eelgrass			0	0	0	0	
Treated	eelgrass	on bed	center - 3	0	25	0	0	
Treated	eelgrass	on bed	center - 3	0	0	0	0	

**Table A7. Imidacloprid concentration in water and eelgrass on Cedar River Bed OB A033 (5 ac) treated with 0.5 lb ai/ac of Nuprid on August 30, 2011 by hand.**

	Sample type	Sample location (on/off bed)	Sample location details & location - #	imidacloprid (ppb)		
				Time of sample		
				2 HAT	54 HAT	102 HAT
Control	water			0	0	0
Treated	water	on bed	center middle -1	1100	0	0
Treated	water	on bed	center high - 2	1400		
Treated	water	off bed	30 m high end NE -3	18		
Treated	water	off bed	30 m high end SE - 4	0.072		
Treated	water	off bed	60 m high end SE - 5	0		
Treated	water	off bed	120 m high end SE -6	0		
Treated	water	off bed	240 m high end SE - 7	0		
Treated	water	off bed	60 m center S - 8	12		
Treated	water	off bed	30 m low end SW - 9	1300		
Treated	water	off bed	30 m high end NW - 10	9.1		
				96 HAT	336 HAT	
Control	eelgrass			0	0	
Treated	eelgrass	on bed	center middle - 1	0	0	
Treated	eelgrass	on bed	center high - 2	0	0	
Treated	eelgrass	off bed	30 m high end SE - 3	0	0	
Treated	eelgrass	off bed	60 m high end SE - 5	0	0	
Treated	eelgrass	off bed	120 m high end SE - 6	0	0	



**Table A8. Imidacloprid concentration in water and eelgrass on Cedar River Bed # OB A101 (4.2 ac) treated with 0.5 lb ai/ac of Mallet on August 31, 2011 by boat application in 12 to 36 inches of water.**

Treatment	Sample type	Sample location (on/off bed)	Sample location details & location - #	imidacloprid (ppb)		
				Time of sample		
				0 to 2 HAT	54 Hat	102 HAT
Control	water			0	0	0
Treated	water	on bed	center middle high - 1	16	0	0
Treated	water	on bed	center high - 2	31		
Treated	water	off bed	30 m low end W - 3	0		
Treated	water	off bed	30 m low end E - 4	0.1		
Treated	water	off bed	30 m high end W - 5	0.25		
Treated	water	off bed	30 m high end N - 6	0.35		
Treated	water	off bed	30 m high end E - 7	0		
				96 hr	336 hr	
Control	eelgrass			0	0	
Treated	eelgrass	on bed	center high - 2	0	0	
Treated	eelgrass	off bed	30 m high end NW - 5	0	0	
Treated	eelgrass	off bed	60 m high end NW - 8	0	0	
Treated	eelgrass	off bed	120 m high end NW - 9	0	0	

**Title:** Impact of imidacloprid on epi-benthic and benthic invertebrates: Initial studies to describe the Sediment Impact Zone (SIZ) related to imidacloprid treatments to manage burrowing shrimp

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**Submitted: To the WGHOGA:** June 26, 2011

**Introduction:** Several studies will be required to meet requirements for the registration and permitting of imidacloprid for use on commercial oyster beds. Among these are: 1) assessments of non-target impacts to the benthic and epibenthic community, fish, and other organisms, 2) sediment degradation studies, and 3) a study of the fate & transport of imidacloprid in the water column. These studies will ultimately be combined to describe the sediment impact zone (SIZ) related to the imidacloprid treatments, as required by Washington State Sediment Management Standards (WAC 173-204-400) and regulated by the Washington State Department of Ecology. These studies are expected to extend over at least two growing seasons. This report presents results of 2010 studies of the impact of imidacloprid on the epi-benthic and benthic invertebrates sampled on two large neighboring beds.

**Objectives:**

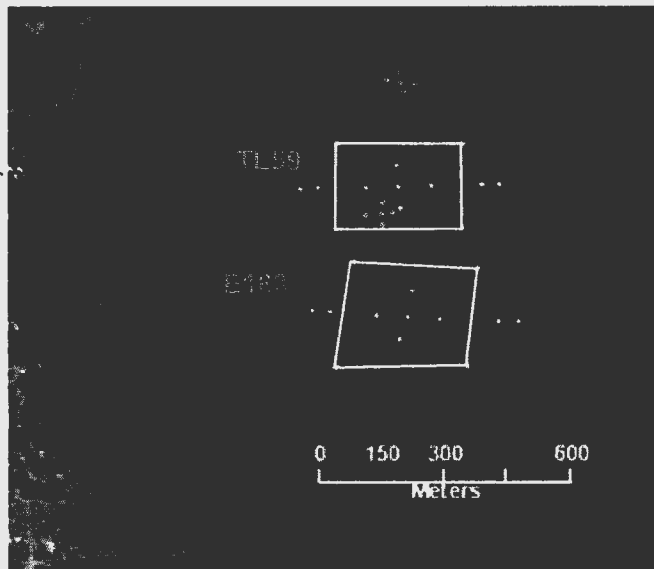
- Measure the impact of imidacloprid on the benthic infauna..
- Apply the data towards the development of a final Sampling and Analysis Plan to describe the Sediment Impact Zone (SIZ) related to applications of imidacloprid to treat burrowing shrimp on commercial shellfish beds.

**Methods:**

Measurements and assessments were made at two of the 19 large plots (>0.1 ac) treated with imidacloprid in 2010. Plots E163 and TL59 were adjacent to one another and of similar sediment types, vegetational cover, elevation, and shrimp burrow density. Both were characterized by mostly non-vegetated sand and were exposed to similar rates and flows of incoming and receding tides. Nuprid 2F was applied at a rate of 2 lb ai/ac to the 10.3 ac E163B site using an ATV on July 10. Mallet 0.5G was applied to the TL59 site using the Fimco Industries battery powered granular spreader mounted on the ATV on July 26. These studies included sampling and analysis for imidacloprid in the water column and pore-water in addition to the sediments and those results are presented in a separate progress report. Non-target impact to salmonids were also assessed at the sites and results are reported elsewhere.

Epibenthic and benthic invertebrates were sampled in association with on-bed samples of pore-water and sediments to be analyzed for imidacloprid concentrations. In each plot, sediments and pore-water were collected along a transect that crossed the bed in the direction of primary tidal inundation (Figure 1). An additional sample was taken on each side of the transect 30 m from the center of the bed.

Benthic invertebrates were sampled at the five on-bed stations at one-day pre-treatment and at 14 and 28 days after treatment. Samples were also collected at nearby untreated sites. Untreated sites for comparison with impact to E163B were sampled only at 1 day before and 14 days after treatment as they were located on TL59, which was treated 14 days after E163B. Samples were collected using a 10.2 cm internal diameter corer to a depth of 10 cm, with each core constituting a replicate. Three replicate core samples were collected per sample site (e.g., 5 sites per sample date). The core was immediately sieved through 0.5 mm mesh using



**Figure 1.** Orientation of 10 ac plots E163 and TL59 and associated sample stations for water, pore-water, sediments (yellow dots), and untreated benthic infauna (blue dots). (Blue dots in TL59 correspond to treatment of E163 2 weeks before treatment of TL59. Treated benthic infauna was sampled at the same in-bed sites as the sediment samples.

salt water, then stored in a 10% buffered formalin solution for 2-4 weeks, then re-sieved through 100  $\mu$ m mesh to remove excess detritus, stained with rose bengal, and stored in 70% isopropyl alcohol. Species identification and enumeration was done by Ruff Wormworks (annelids), or PSI staff (crustaceans and mollusks).

The primary tactic to determine impact of imidacloprid was by direct comparison of three primary descriptors (absolute abundance, taxonomic richness, and Shannon-Wiener diversity of organisms) within each of three primary taxons (Class Crustacea, Class Polychaeta, and Phylum Mollusca) on beds treated with imidacloprid to untreated beds (reference or check beds). An adverse affect was determined to occur when abundance or richness on treated was <50% of the mean values on the untreated bed. Statistical analyses featured a preliminary Shapiro-Wilk test for normality of data (95% C.I.) followed by t-tests ( $\alpha=0.05$ ) at each sample interval. Non-normally distributed data were transformed (arcsine or Box-Cox transformations) prior to analysis by t-test.

This test for adverse determination assumes that the primary descriptors did not differ substantially between treated and untreated beds prior to treatment. The duration of any adverse affect can consequently be measured by comparing the descriptors' values on treated and untreated at multiple post-treatment intervals.

However, abundance, richness, and diversity of primary taxa were not always the same on treated and untreated beds prior to treatment. Such an inherent and unforeseen discrepancy between treated and untreated beds can be resolved by comparing the change in the proportions of the primary descriptors on the treated bed over time. If the proportions do not change substantially or significantly after treatment, impact can be assumed to be minimal. If the proportions decline substantially after treatment, the impact can be assumed to be correspondingly greater. Note that a proportion of <33% is equivalent to the ratio of <50% that was used in the primary comparison, as described above. Change in the proportions of abundance, richness, and diversity provide a better assessment than change in their ratios on treated to untreated, as the latter sometimes involved dividing by zero, resulting in missing values and biasing the results. The proportions can be calculated two different ways: 1) as a proportion of the means of replicates in treated and untreated beds ( $\Sigma T/N / (\Sigma T/N + \Sigma UT/N)$ , where T is Treated, UT is untreated, and N is the number of samples) and, 2) as the mean of the proportions in treated and untreated replicates from each bed ( $\Sigma ((T/(T+UT))/N)$ , where T is treated, UT in untreated, and N is the number of samples). The solutions to these different computational methods are not equivalent due to the Distributive Law of Arithmetic. For example, given the hypothetical numbers of organisms/sample on the treated bed and untreated beds as 2,3, and 4 and 2, 4, 6, respectively, the first method gives a proportion on the treated bed of 0.428 ( $((2+3+4)/3 \div ((2+3+4)/3 + (2+4+6)/3))$ ) while the second method a proportion of 0.44 ( $((2/(2+2)+3/(3+4)+4/(4+6))/3)$ ). The former is most directly and easily comparable to the 50% ratio assessment used as the primary indicator of adverse affect, but the latter allows for the computation of variance about the mean and associated statistical analyses. Proportions were transformed to arcsine values prior to statistical analysis (t-test or oneway analysis of variance ( $\alpha=0.05$ )).

An additional analysis compared the relationships between the abundance, richness, and diversity of polychaetes, mollusks, and crustaceans to the concentrations of imidacloprid measured in pore-water sampled. Six separate regression models were tested for their ability to describe the relationship between each descriptor and the average concentrations of imidacloprid sampled at 7 post treatment intervals from each of 4 on-bed sample stations where the invertebrates were sampled (see Grew et al., 2011 for pesticide sampling and analytical methods). Linear, logarithmic, inverse, power, s, and exponential regressions were fit to benthic data sampled on Bed E163 at 15 days after treatment and to benthic data sampled on Bed TL59 at 14 and at 28 days after treatment using CURVE-FIT (SPSS V14.0) (F-test,  $\alpha=0.05$ ,  $R^2 > 0.9$ ).

Finally, a power analysis of the results was conducted to establish a the most powerful, precise, and



logistically available sample size for future studies (Appendix B).

### Results:

63 organisms were identified and counted, 33 to species, 9 to genus, 4 to family, 2 to infraorder, 1 to suborder, 6 to order, 6 to class, 1 to sub-phylum and 1 to phylum (Table 1).

The three primary taxa (Polychaetes, Mollusks, and Crustaceans) comprised 97.9% of all organisms sampled throughout the course of the study. Crustaceans comprised the epibenthic fauna whereas all others are benthic organisms.

Table 1. List of 61 taxa identified from samples taken from Beds E163 and TL59 before and after treatment with imidacloprid in 2010 and associated untreated beds.			
Phylum Annelida			
Class Polychaeta			
Order Phyllodoidea			
Family Syllidae			
Sphaerosyllis californiensis	01	Rhynchospio glutaea	25
Sphaerosyllis sp(p)	02	Spionidae, unident (post-larval)	26
Exogone dwisula	03	Order Cirratulida	
Family Nereididae		Family Cirratulidae	
Platynereis bicanaliculata	04	Aphelochaeta monilaris	27
Platynereis sp. (juv)	05	Tharyx parvus	28
Family Nephtyidae		Order Opheliida	
Nephtys caeca	06	Family Opheliidae	
Nephtys sp. indet. (juv)	07	Armandia brevis	29
Family Goniadidae		Order Capitellida	
Glycinde picta	08	Family Capitellidae	
Glycinde sp. (juv)	09	Mediomastus californiensis	30
Family Hesionidae		Notonastus tenuis	31
Microphthalmus sp.	10	Class Oligochaeta	32
Micropodarke dubia	11	Phylum Mollusca	
Family Phyllodoidea		Class Gastropoda	
Eteone californica	12	Unidentified (juv)	33
Eteone fauchaldia	13	Unidentified (adult)	34
Eteone sp. (juv)	14	Order Neotaenioglossa	
Order Orbiniida		Family Littorinidae	
Family Orbiniidae		Lacuna variegata	35
Leitoscoloplos sp. (juv)	15	Order Neogastropoda	
Paronella platybranchia	16	Family Nassariidae	
Scoloplos armiger armiger	17	Ilyanassa obsoleta	36
Scoloplos armiger alaskensis	18	Class Bivalvia	
Scoloplos sp. (juv)	19	Unidentified (juv)	37
Order Spionida		Unidentified (adult)	38
Family Spionidae		Subclass Heterodonta	
Scolecopsis squamata	20	Family Mytilidae	
Polydora cornuta	21	Unidentified Mytilid (juv)	39
Pseudopolydora kemp	22		
Pseudopolydora paucibranchiata	23		
Pygospio elegans	24		
		Family Cardiidae	
		Clinocardium nuttali	40
		Family Veneridae	
		Prothaca staminea	41
		Tapes philippinarum	42
		Family Myidae	
		Unidentified Myid	43
		Sphenia ovoidea	44
		Cryptomya californica	45
		Mya truncata	46
		Mya sp.	47
		Family Tellinidae	
		Macoma balthica	48
		Macoma inquinata	49
		Macoma nasuta	50
		Macoma sp.	51
		Phylum Nemertea	52
		Phylum Arthropoda – Sub Phylum Crustacea	
		Unidentified crustacean	53
		Class Malacostraca	
		Order Tanaidacea	54
		Order Cumacea	55
		Order Amphipoda	
		Suborder Gammaridea	56
		Suborder Corophidea	
		Infraorder Caprellida	57
		Infraorder Corophida	58
		Order Decapoda	59
		Family Pasiphaeidae	60
		Class Ostracoda	
		Order Ostracoda	61
		Class Copepoda	
		Order Calanoida	62
		Order Harpacticoida	63

Abundance, richness, and diversity of polychaetes was <50% lower on the treated than on the untreated plots at all sample dates and times except at Bed E163 at 15 days after treatment, where abundance was 47.3% on the treated compared to the untreated bed (Table 2). The three descriptors were nearly equivalent on treated and untreated beds for mollusks. Although richness and diversity of crustaceans were below 50% on the treated than on the untreated bed in only one instance, they were always much less abundant on the treated bed, even before treatment.

Table 2. Absolute Abundance, Taxonomic Richness, and Shannon-Weiner Diversity ( $\bar{x} \pm \text{S.E.}$ ) of three primary taxonomic groups at Bed E163 treated with 2.0 lb a.i./ac liquid imidacloprid on July 10 and at Bed TL 59 treated with 0.5 lb granular imidacloprid on July 26 (Imid) and respective nearby untreated beds (Check). DAT, Days After Treatment. Letters following values indicate a significant difference between treated and untreated (t-test,  $P=0.05$ ). Bolded values indicate that levels in treatment plot are  $< 50\%$  of levels in untreated plot. Asterisks (\*) indicate square-root transformation:  $Y = (Y+1)^{1/2}$ ; other footnotes indicate Box-Cox data transformation:  $Y = (Y^{\lambda} - 1) / \lambda$ , where  $Y > 0$ .

Taxon	Bed	DAT	Treatment	Abundance	Richness	Diversity
Polychaetes	E 163	-1	Imid	23.5 $\pm$ 3.2 a †	7.0 $\pm$ 0.4 ††	1.6 $\pm$ 0.1 b
			Check	37.9 $\pm$ 5.7 b †	7.2 $\pm$ 0.4 ††	1.4 $\pm$ 0.1 a
		15	Imid	<b>31.0 <math>\pm</math> 3.1 a</b>	8.5 $\pm$ 0.3 a	1.7 $\pm$ 0.1
			Check	<b>65.5 <math>\pm</math> 6.6 b</b>	10.7 $\pm$ 0.8 b	1.7 $\pm$ 0.1
	TL 59	-1	Imid	65.5 $\pm$ 6.6	10.7 $\pm$ 0.8	1.7 $\pm$ 0.1 b
			Check	60.5 $\pm$ 4.1	10.5 $\pm$ 0.4	1.8 $\pm$ 0.1 a
		14	Imid	56.7 $\pm$ 5.6	11.2 $\pm$ 0.5	1.9 $\pm$ 0.1
			Check	64.2 $\pm$ 5.4	12.1 $\pm$ 0.6	2.0 $\pm$ 0.1
Mollusks	E 163	-1	Imid	1.95 $\pm$ 0.4 ‡	1.2 $\pm$ 0.2 †	0.2 $\pm$ 0.1
			Check	1.00 $\pm$ 0.2 ‡	0.8 $\pm$ 0.2 †	0.1 $\pm$ 0.1
		15	Imid	27.8 $\pm$ 4.6 *	1.8 $\pm$ 0.2 †	0.2 $\pm$ 0.1 ††
			Check	34.9 $\pm$ 5.8 *	2.2 $\pm$ 0.2 †	0.2 $\pm$ 0.1 ††
	TL 59	-1	Imid	34.9 $\pm$ 5.7	2.2 $\pm$ 0.8 ††	0.2 $\pm$ 0.1 ††
			Check	44.2 $\pm$ 3.9	2.1 $\pm$ 0.8 ††	0.2 $\pm$ 0.1 ††
		14	Imid	37.8 $\pm$ 6.3	3.1 $\pm$ 0.2 f	0.5 $\pm$ 0.1 ff
			Check	40.4 $\pm$ 4.3	3.2 $\pm$ 0.2 f	0.4 $\pm$ 0.1 ff
Crustaceans	E 163	-1	Imid	7.1 $\pm$ 1.1 a *	2.3 $\pm$ 0.3 *	0.6 $\pm$ 0.1 *
			Check	<b>14.9 <math>\pm</math> 3.0 b *</b>	3.0 $\pm$ 0.3 *	0.7 $\pm$ 0.1 *
		15	Imid	<b>5.8 <math>\pm</math> 0.9 a *</b>	<b>2.4 <math>\pm</math> 0.3 a *</b>	0.7 $\pm$ 0.1 a
			Check	<b>47.3 <math>\pm</math> 8.4 b *</b>	<b>5.1 <math>\pm</math> 0.3 b *</b>	1.2 $\pm$ 0.1 b
	TL 59	-1	Imid	<b>47.3 <math>\pm</math> 8.4 a *</b>	5.1 $\pm$ 0.3 §§	1.3 $\pm$ 0.1
			Check	<b>126.5 <math>\pm</math> 13.2 b *</b>	4.9 $\pm$ 0.3 §§	1.2 $\pm$ 0.1
		14	Imid	<b>41.6 <math>\pm</math> 7.8 a §</b>	4.8 $\pm$ 0.2 ¥	1.3 $\pm$ 0.1 £
			Check	<b>128.6 <math>\pm</math> 15.9 b §</b>	5.0 $\pm$ 0.2 ¥	1.3 $\pm$ 0.1 £
		28	Imid	<b>89.4 <math>\pm</math> 20.8 f</b>	4.5 $\pm$ 0.2 a ¥¥	1.2 $\pm$ 0.1 a
			Check	<b>214.7 <math>\pm</math> 58.1 f</b>	5.4 $\pm$ 0.2 b ¥¥	1.3 $\pm$ 0.1 b

†, = 0.3

‡, = -0.3

f, = 0.2

§, = 0.4

¥, = 1.6

£, = 4.9

††, = 0.8

‡‡, = -2.0

ff, = -1.8

§§, = 0.5

¥¥, = 1.9

The proportions of abundance, richness, and diversity of each of the three major groups was often lower, and twice was  $< 50\%$  (i.e., crustacean abundance at both beds) on the treated compared to the untreated bed before treatment. Proportion of crustacean abundance on Bed E163 (treated with liquid imidacloprid @ 2 lb a.i./ac) declined by more than 50% at 15 days after treatment than at the day before treatment. The proportion of molluscan diversity on the treated bed (computed as the mean of the proportions in all replicates) was  $< 33\%$  before treatment but was 40% at 15 days after treatment. The proportions of all other descriptors on the treated beds were  $> 33\%$  both before and after treatment.

Table 3. Proportions of Abundance, Taxonomic Richness, and Shannon Diversity of three primary taxons on treated (Bed E163 with 2.0 lb a.i./ac liquid imidacloprid; Bed TL59 with 0.5 lb a.i./ac granular imidacloprid) on both treated and untreated beds, as calculated two different ways. DAT, Days After Treatment.

Taxon	Bed	DAT	Proportions of Means			Means of Proportions		
			Abundance	Richness	Diversity	Abundance	Richness	Diversity
Polychaetes	E 163	-1	38.3	49.1	54.2	40.9 ± 5.0	49.1 ± 2.6	54.2 ± 2.0
		15	31.6	44.7	49.8	34.5 ± 3.8	44.6 ± 2.2	49.0 ± 1.6
	TL 59	-1	52.0	50.4	48.8	50.5 ± 3.5	49.8 ± 1.9	48.1 ± 2.2
		14	46.9	48.2	48.4	46.9 ± 3.6	48.4 ± 1.6	48.4 ± 1.1
		28	52.8	49.2	48.1	55.0 ± 3.6	49.4 ± 1.8	49.4 ± 2.0
Mollusks	E 163	-1	66.1	57.5	65.3	56.4 ± 8.1	54.2 ± 7.8	20.6 ± 8.6
		15	47.3	45.0	39.4	43.7 ± 7.0	45.5 ± 3.8	40.3 ± 9.6
	TL 59	-1	44.1	51.5	60.1	38.7 ± 4.8	51.0 ± 3.6	53.1 ± 9.0
		14	48.3	49.6	54.7	45.5 ± 3.9	49.6 ± 1.8	54.7 ± 2.5
		28	41.2	46.7	44.2	37.7 ± 4.0	46.5 ± 1.4	43.3 ± 2.1
Crustaceans	E 163	-1	32.3	43.4	44.2	37.4 ± 5.5 b	41.6 ± 4.6	37.8 ± 7.4
		15	11.0	32.9	36.2	13.0 ± 2.8 a	29.7 ± 3.8	29.9 ± 4.9
	TL 59	-1	27.2	50.9	51.6	27.7 ± 3.6	50.6 ± 2.5	51.6 ± 1.0
		14	24.4	48.7	49.9	25.0 ± 3.3	48.6 ± 1.3	49.7 ± 1.0
		28	29.4	45.3	47.7	30.9 ± 5.4	45.1 ± 1.8	47.7 ± 1.2

Only 3 of the 54 best-fit analyses of abundance, richness, and diversity of polychaetes, mollusks, and crustaceans were significant (Tables A1 – A3, Appendix A). An “S” curve significantly predicted the richness of crustaceans on Bed E163 and 15 days after treatment. The diversity of crustaceans at the same sample bed and date was inversely related to imidacloprid concentration; diversity was higher at stations with high concentrations of imidacloprid. That relationship could be described by both linear and logarithmic models with reasonable precision.

### Discussion

The often large disparities between crustacean on treated and untreated beds, both before and after treatment with imidacloprid, was likely a consequence differing densities of algae and eelgrass between the beds. Unfortunately, algae and eelgrass densities were not closely monitored. All of the crustaceans sampled in this study are strongly associated with (living and/or feeding on) plant materials. Decapods, some of which are filter feeders or scavengers and so do not depend on plants as strongly as the other crustaceans, accounted for ~0.06% of all sampled crustaceans.

The lack of imidacloprid impact on the benthic invertebrates was also indicated by a corresponding lack of almost any significant relationship between imidacloprid concentration and the abundance, richness, and diversity of polychaetes, mollusks, and crustaceans. The inverse relationship between imidacloprid concentration and crustacean diversity at E163 at 15 days after treatment is counter-intuitive and, despite the significant F value, likely represents a coincidental phenomenon.

These results generally agree with those of previous studies that have demonstrated that the impact of imidacloprid (Booth et al. 2011a, Booth et al. 2011b) and carbaryl (Dumbauld et al 2001, Ferraro and Cole 2007, Booth 2006, Booth 2008) on the epi-benthic and benthic invertebrates is negligible compared to seasonal and other natural events on the development of estuarine species, populations, and communities.

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## Appendix A.

Table A1. Statistics and parameter estimates of 6 regression models fitted to the absolute abundance, taxonomic richness, and Shannon diversity for each of three primary taxa at Bed E163 at 14 days after treatment (DAT) with 2.0 lb a.i./ac liquid imidacloprid.

Bed	DAT	Descriptor	Taxon	Regression	Model Summary					Parameter Estimates	
					R <sup>2</sup>	F	df1	df2	Sig.	Constant	b1
E163	14	Abundance	Polychaetes	Linear	.298	.848	1	2	.454	1185	-23
				Logarithmic	.234	.611	1	2	.516	2575	-628
				Inverse	.160	.382	1	2	.599	-93	14752
				Power	.519	2.156	1	2	.280	278660	-2
				S	.424	1.473	1	2	.349	3	54
				Exponential	.591	2.888	1	2	.231	2336	0
			Mollusks	Linear	.093	.204	1	2	.695	686	-11
				Logarithmic	.168	.404	1	2	.590	1645	-401
				Inverse	.305	.876	1	2	.448	-282	13061
				Power	.017	.034	1	2	.870	516	0
				S	.083	.182	1	2	.711	5	15
				Exponential	.000	.001	1	2	.983	225	0
			Crustaceans	Linear	.448	1.626	1	2	.330	54	0
				Logarithmic	.655	3.802	1	2	.191	115	-19
				Inverse	.678	4.215	1	2	.176	15	977
				Power	.456	1.675	1	2	.325	300	-1
				S	.523	2.193	1	2	.277	3	30
				Exponential	.252	.673	1	2	.498	47	0
		Richness	Polychaetes	Linear	.471	1.781	1	2	.314	4192	-452
				Logarithmic	.495	1.961	1	2	.296	8731	-3925
				Inverse	.519	2.161	1	2	.279	-3675	33881
				Power	.646	3.647	1	2	.196	379206231304	-10
				S	.674	4.141	1	2	.179	-5	87
				Exponential	.617	3.222	1	2	.214	3398877	-1
			Mollusks	Linear	.049	.103	1	2	.779	103	-21
				Logarithmic	.057	.121	1	2	.761	115	-70
				Inverse	.066	.141	1	2	.743	-36	224
				Power	.013	.026	1	2	.887	110	-1
				S	.018	.036	1	2	.867	2	4
				Exponential	.009	.017	1	2	.907	80	0
			Crustaceans	Linear	.627	3.369	1	2	.208	2306	-788
				Logarithmic	.673	4.121	1	2	.179	2030	-1897
				Inverse	.721	5.159	1	2	.151	-1536	4459
				Power	.869	13.310	1	2	.068	12747	-5
				S	.904	18.813	1	2	.049	0	11
				Exponential	.832	9.886	1	2	.088	27424	-2
		Diversity	Polychaetes	Linear	.276	.761	1	2	.475	3596	-1934
				Logarithmic	.292	.824	1	2	.460	2037	-3284
				Inverse	.308	.892	1	2	.445	-2985	5550
				Power	.114	.257	1	2	.663	2059	-5
				S	.128	.293	1	2	.642	0	8
				Exponential	.101	.223	1	2	.683	15828	-3
			Mollusks	Linear	.002	.005	1	2	.951	517	-433
				Logarithmic	.002	.004	1	2	.953	577	83
				Inverse	.021	.043	1	2	.855	710	-48
				Power	.001	.002	1	2	.971	264	0
				S	.021	.042	1	2	.857	6	0
				Exponential	.007	.013	1	2	.919	294	-2
			Crustaceans	Linear	.919	22.720	1	2	.041	1876	-2219
				Logarithmic	.970	65.645	1	2	.015	-165	-1175
				Inverse	.988	162.583	1	2	.006	-581	550
				Power	.881	14.824	1	2	.061	60	-3
				S	.868	13.096	1	2	.069	3	1
				Exponential	.870	13.429	1	2	.067	5077	-5

Table A2. Statistics and parameter estimates of 6 regression models fitted to the absolute abundance, taxonomic richness, and Shannon diversity for each of three primary taxa at Bed TL59 at 14 days after treatment (DAT) with 0.5 lb a.i./ac liquid imidacloprid.

Bed	DAT	Descriptor	Taxon	Regression	Model Summary					Parameter Estimates	
					R <sup>2</sup>	F	df1	df2	Sig.	Constant	b1
TL59	14	Abundance	Polychaetes	Linear	.525	3.312	1	3	.166	-23	1
				Logarithmic	.457	2.525	1	3	.210	-166	50
				Inverse	.383	1.862	1	3	.266	77	-2324
				Power	.355	1.651	1	3	.289	0	2
				S	.282	1.178	1	3	.357	5	-68
				Exponential	.423	2.200	1	3	.235	5	0
			Mollusks	Linear	.581	4.158	1	3	.134	63	-1
				Logarithmic	.571	3.998	1	3	.139	121	-25
				Inverse	.501	3.014	1	3	.181	13	553
				Power	.621	4.914	1	3	.113	615	-1
				S	.480	2.770	1	3	.195	3	19
				Exponential	.717	7.591	1	3	.070	84	0
			Crustaceans	Linear	.075	.163	1	2	.726	829	-68
				Logarithmic	.031	.063	1	2	.825	827	-233
				Inverse	.005	.010	1	2	.929	345	449
				Power	.207	.524	1	2	.544	2154	-1
				S	.131	.303	1	2	.637	4	5
				Exponential	.286	.802	1	2	.465	1220	0
		Richness	Polychaetes	Linear	.216	.824	1	3	.431	-72	9
				Logarithmic	.217	.831	1	3	.429	-217	103
				Inverse	.217	.832	1	3	.429	135	-1130
				Power	.180	.659	1	3	.476	0	3
				S	.176	.641	1	3	.482	6	-35
				Exponential	.183	.673	1	3	.472	1	0
			Mollusks	Linear	.669	4.050	1	2	.182	4101	-2022
				Logarithmic	.703	4.735	1	2	.162	2565	-3623
				Inverse	.736	5.564	1	2	.142	-3160	6426
				Power	.449	1.633	1	2	.330	9976	-7
				S	.483	1.866	1	2	.305	-1	12
				Exponential	.416	1.427	1	2	.355	143707	-4
			Crustaceans	Linear	.052	.164	1	3	.713	-40	15
				Logarithmic	.061	.195	1	3	.688	-92	80
				Inverse	.071	.230	1	3	.665	121	-417
				Power	.081	.263	1	3	.643	0	3
				S	.091	.300	1	3	.622	7	-16
				Exponential	.071	.229	1	3	.665	1	1
		Diversity	Polychaetes	Linear	.166	.596	1	3	.496	146	-60
				Logarithmic	.168	.604	1	3	.494	103	-113
				Inverse	.169	.612	1	3	.491	-79	209
				Power	.060	.190	1	3	.692	114	-2
				S	.061	.196	1	3	.688	1	4
				Exponential	.058	.185	1	3	.696	268	-1
			Mollusks	Linear	.343	1.563	1	3	.300	-5	73
				Logarithmic	.359	1.679	1	3	.286	60	39
				Inverse	.372	1.778	1	3	.275	74	-19
				Power	.390	1.920	1	3	.260	71	1
				S	.395	1.958	1	3	.256	5	-1
				Exponential	.378	1.824	1	3	.270	7	3
			Crustaceans	Linear	.191	.709	1	3	.461	-153	138
				Logarithmic	.185	.683	1	3	.469	-22	185
				Inverse	.179	.656	1	3	.477	218	-248
				Power	.124	.425	1	3	.561	6	5
				S	.117	.398	1	3	.573	8	-7
				Exponential	.131	.451	1	3	.550	0	4

Table A3. Statistics and parameter estimates of 6 regression models fitted to the absolute abundance, taxonomic richness, and Shannon diversity for each of three primary taxa at Bed TL59 at 28 days after treatment (DAT) with 0.5 lb a.i./ac liquid imidacloprid.

Bed	DAT	Descriptor	Taxon	Regression	Model Summary					Parameter Estimates	
					R <sup>2</sup>	F	df1	df2	Sig.	Constant	b1
TL59	28	Abundance	Polychaetes	Linear	.188	.463	1	2	.566	-118	2
				Logarithmic	.183	.449	1	2	.572	-622	158
				Inverse	.179	.435	1	2	.577	198	-10453
				Power	.177	.430	1	2	.579	0	5
				S	.173	.418	1	2	.584	9	-364
				Exponential	.181	.441	1	2	.575	0	0
			Mollusks	Linear	.467	1.751	1	2	.317	65	-1
				Logarithmic	.422	1.458	1	2	.351	91	-16
				Inverse	.383	1.243	1	2	.381	25	246
				Power	.315	.921	1	2	.438	155	0
				S	.298	.849	1	2	.454	3	8
				Exponential	.325	.964	1	2	.430	69	0
			Crustaceans	Linear	.315	1.382	1	3	.324	56	-1
				Logarithmic	.327	1.456	1	3	.314	113	-22
				Inverse	.324	1.435	1	3	.317	10	742
				Power	.334	1.506	1	3	.307	422	-1
				S	.341	1.553	1	3	.301	3	26
				Exponential	.302	1.298	1	3	.337	57	0
		Richness	Polychaetes	Linear	.101	.225	1	2	.682	106	-6
				Logarithmic	.119	.269	1	2	.656	210	-71
				Inverse	.136	.315	1	2	.631	-36	833
				Power	.061	.131	1	2	.752	2446	-2
				S	.075	.163	1	2	.726	1	22
				Exponential	.048	.101	1	2	.780	160	0
			Mollusks	Linear	.673	6.184	1	3	.089	180	-47
				Logarithmic	.693	6.783	1	3	.080	201	-149
				Inverse	.713	7.465	1	3	.072	-120	470
				Power	.645	5.451	1	3	.102	6934	-5
				S	.661	5.838	1	3	.094	-2	15
				Exponential	.629	5.094	1	3	.109	3411	-2
			Crustaceans	Linear	.626	3.341	1	2	.209	165	-28
				Logarithmic	.640	3.557	1	2	.200	229	-128
				Inverse	.649	3.703	1	2	.194	-91	569
				Power	.448	1.622	1	2	.331	8791	-4
				S	.463	1.726	1	2	.319	0	17
				Exponential	.428	1.497	1	2	.346	1274	-1
		Diversity	Polychaetes	Linear	.181	.441	1	2	.575	104	-36
				Logarithmic	.204	.513	1	2	.548	79	-67
				Inverse	.226	.584	1	2	.525	-30	123
				Power	.190	.470	1	2	.564	124	-2
				S	.206	.518	1	2	.546	1	4
				Exponential	.173	.418	1	2	.584	308	-1
			Mollusks	Linear	.482	1.861	1	2	.306	-36	107
				Logarithmic	.509	2.071	1	2	.287	64	66
				Inverse	.531	2.265	1	2	.271	98	-39
				Power	.704	4.755	1	2	.161	91	3
				S	.726	5.292	1	2	.148	6	-2
				Exponential	.677	4.198	1	2	.177	1	4
			Crustaceans	Linear	.015	.030	1	2	.879	-43	66
				Logarithmic	.017	.035	1	2	.869	21	88
				Inverse	.020	.041	1	2	.858	132	-115
				Power	.097	.215	1	2	.689	8	7
				S	.103	.230	1	2	.679	11	-9
				Exponential	.091	.200	1	2	.698	0	6

### Appendix B

#### Sufficient sample size to assess impacts to the epibenthic and benthic invertebrates: A power analysis of 2010 Willapa Bay Samples

In a 2010 study of the impact of imidacloprid on benthic invertebrates, 16 or 20 core-replicates were taken among 5 sample sites on both treated and untreated beds at pre and post treatment intervals (Booth and Rasmussen 2011). Organisms in each core-replicate sample were identified, mostly to species, and counted. The Absolute Abundance (number of individuals), Taxonomic Richness (number of species or otherwise most precise taxonomic unit) of benthic invertebrates were compared among treated and untreated beds at pre and post treatment intervals using t-tests.

This appendix to that study addresses the sample sizes (number of core-replicates) used, with an objective to establish a the most powerful, precise, and logistically available sample size for future studies.

Power analysis was conducted on the Absolute Abundance and Taxonomic Richness of each of Class Polychaetae, Phylum Mollusca, and Class Crustacea (Table 1). The one-tailed t-test analyses ( $\alpha = 0.05$ ) compared the mean values of each descriptor to the test criteria for an adverse affect (i.e., 50% of the mean value) as measured on the untreated beds associated with simultaneous sampling of the two beds treated with imidacloprid (IPM SPSS Sample Power™, Release 3.0). The effect size for all analyses was calculated as the difference between the mean and the test value divided by the standard deviation and, as stated in the reports that Sample Power generated for each analysis, “represented the smallest effect that would be important to detect, as any smaller effect would not be of substantive significance”. Analyses outputs were the Power, Precision, and number of samples needed to reject the null hypothesis (one-tailed t-test,  $\alpha = 0.05$ , power threshold = 80%). All analyses were conducted at a 3 decimal level of precision.

Table 1. Power, Precision, (Confidence interval for t-tests), and predicted number of samples required to obtain reject the null hypothesis that mean abundance or mean richness was significantly less than the test value ( $\frac{1}{2}$  mean) ( $N_{80}$ ), as measured on the untreated bed associated with each of two treated beds (E163 and TL59) and two sample dates\*. N, actual number of samples; S.D., standard deviation. , predicted number of samples needed to between the mean and

bed	date	Taxon	Abundance							Richness						
			N	Mean	SD	Test	Power(%)	Precision	$N_{80}$	N	Mean	SD	Test	Power(%)	Precision	$N_{80}$
E163	7/9/2011	polychaetes	20	37.9	25.4	19.0	99.4	5.0	13	20	7.3	2.0	3.6	99.9	0.8	4
		mollusks	20	1.0	1.0	0.5	69.5	0.4	28	20	0.9	0.7	0.4	86.1	0.3	17
		crustaceans	20	14.9	13.2	7.5	78.9	5.0	21	20	3.0	1.5	1.5	99.8	0.6	8
E163	7/25/2011	polychaetes	16	65.5	26.5	32.8	99.9	11.4	6	16	10.7	3.0	5.3	99.9	1.1	4
		mollusks	16	34.9	23.1	17.5	94.7	8.8	13	16	2.2	0.8	1.1	99.9	0.3	6
		crustaceans	16	47.3	33.5	23.7	91.9	12.7	14	16	5.1	1.3	2.5	99.9	0.5	4
TL59	8/9/2011	polychaetes	20	64.2	24.3	32.1	99.9	9.3	6	20	12.1	2.6	6.1	99.9	1.0	3
		mollusks	20	40.4	19.4	20.2	99.8	7.4	8	20	3.2	0.8	1.6	99.9	0.3	4
		crustaceans	20	128.7	71.0	64.3	98.8	27.0	10	20	5.1	0.8	2.5	99.9	0.3	3
TL59	8/23/2011	polychaetes	16	58.3	31.5	29.1	99.7	13.5	9	16	11.9	2.4	5.9	99.9	1.0	3
		mollusks	16	54.3	20.6	27.2	99.9	8.8	6	16	3.5	0.5	1.8	99.9	0.2	3
		crustaceans	16	214.7	232.3	107.3	54.8	99.5	31	16	5.4	0.6	2.7	99.9	0.3	3

\* The same bed was used as an untreated comparison to the treated E163 bed and the pre-treatment for bed TL59 on July 25.

Power was above the standard acceptable level of 80% in 21 of the 24 analyses, indicating that sample sizes were sufficient. In the 3 cases where Power was less than 80%, Sample Power predicted that sample sizes of 28, 21, and 31 would be sufficient.

The number of sample replicates (16 or 20 per sample date/plot) was sufficient to test the null hypothesis that the mean abundance or richness of the polychaetes, mollusks, or crustaceans was significantly less than one half of that mean value (the test criteria for adverse affect) in 21 of the 24 analyses (one-tailed t-test,  $\alpha$

= 0.05). Power of analysis was > 99% in 17 analyses. The 3 analyses with insufficient sample sizes yielded predicted sample sizes of 21, 28, and 31 (power = 80%). The last of these analyses involved an untreated bed with inordinately high abundance of crustaceans, very likely due to late season growth of eelgrass. The analysis which predicted a necessary sample size of 28 involved exceptionally low abundance of mollusks. Aside from these anomalies, the power analysis predicts that a sample size of 24 core-replicates will be sufficient for the studies proposed here.





**Draft  
Sampling and Analysis Plan  
Experimental Trials for  
Imidacloprid Use in Willapa Bay  
Willapa Bay, Washington**



**Prepared for  
Willapa Grays Harbor Oyster  
Growers Association**



**March 16, 2012  
12733-02**



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Willapa Grays Harbor Oyster Growers Association***

***March 16, 2012  
12733-02***

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**DRAFT  
SAMPLING AND ANALYSIS PLAN  
EXPERIMENTAL TRIALS FOR IMIDACLOPRID USE IN WILLAPA BAY  
WILLAPA BAY, WASHINGTON**

**1.0 INTRODUCTION AND BACKGROUND INFORMATION**

This Sampling and Analysis Plan (SAP) addresses sediment porewater, water column, vegetation, and benthic infauna investigations in Willapa Bay and Grays Harbor, Washington (Figure 1). These investigations will be conducted to determine the efficacy, environmental fate and transport, and potential for impacts on non-target species of the pesticide imidacloprid. The ultimate goal is to describe the sediment impact zone (SIZ) related to the potential commercial use of imidacloprid to manage burrowing shrimp in commercial oyster and clam beds in Willapa Bay and Grays Harbor.

This SAP was prepared in conjunction with other activities related to registration and NPDES permitting of imidacloprid for use in these estuaries, particularly with regard to an Application for SIZ Authorization by Washington State Department of Ecology (Ecology). Accordingly, it was prepared with input from that agency and under guidance of the Sediment Sampling and Analysis Plan Appendix (Ecology 2008) as well as Chapters 173-204 WAC, the Washington State Sediment Management Standards (Ecology 1995).

**1.1 Site Background and History**

Indigenous people have collected shellfish in Willapa Bay for thousands of years. Oysters and clams have been farmed in Willapa Bay since about 1849. Willapa Bay and Grays Harbor are currently home to thousands of acres of commercial oyster and clam beds. Ghost shrimp (*Neotrypaea californiensis*) and mud shrimp (*Upogegia pugettensis*; collectively burrowing shrimp) disrupt commercial shellfish culture by destabilizing the sediments on commercial shellfish beds, causing significant mortality and reduced growth rates. This threatens the viability of the entire commercial shellfish industry on the coast of Washington State. As part of an ongoing integrated pest management program, imidacloprid has been under investigation as a potential replacement for carbaryl, a carbamate insecticide that has been used to control burrowing shrimp in Willapa Bay and Grays Harbor for more than 60 years.

Research on imidacloprid as a control agent for burrowing shrimp on commercial shellfish beds in Willapa Bay and Grays Harbor has been ongoing



since 2008. From 2008 to 2011 several large scale trials were conducted. Results of the 2010 trials featured both small (<0.1 acre) and large (>1 acre) plots and generally indicated that the granular and liquid forms of imidacloprid applied at 0.5 pound (lb) active ingredient per acre (a.i./ac) were moderately to highly effective in reducing burrowing shrimp densities. These studies included sampling and analysis of imidacloprid in the water column, sediment pore-water, and whole sediments, as well as studies of the impact to the epibenthic and benthic invertebrates. Studies planned for 2012 would build on this previous work with the goal of better defining the efficacy, fate and transport, and impacts to non-target organisms of imidacloprid applications to oyster and clam beds in Willapa Bay and Grays Harbor.

## **1.2 Regulatory Framework**

The regulatory basis to determine new pesticide compliance with the Sediment Management Standards (SMS) requires a permittee to:

- 1) Demonstrate that there are no effects on biological resources, or
- 2) Apply for a Sediment Impact Zone (SIZ) and demonstrate that the discharge does not cause more than minor adverse effects on biological resources.

If a SIZ is required, the permittee must demonstrate that the other requirements of the SIZ are met, including best management practices and all known, available and reasonable methods of prevention, control, and treatment to reduce the discharge. The permittee needs to ensure that Integrated Pest Management practices are implemented as much as possible to reduce impacts to non-target organisms.

In order to meet these requirements this SAP describes the procedures to be employed to evaluate if there are impacts imidacloprid application on biological resources and, if impacts are observed, to determine the spatial and temporal magnitude of the SIZ.



## **1.3 Summary of Existing Data**

### **1.3.1 Imidacloprid Properties and Environmental Fate**

#### ***Physical and Chemical Properties***

Imidacloprid (CAS number 138261-41-3) is a chloronicotiny/neonicotinoid pesticide. It has a molecular weight of 255.7 and is a colorless to cream-colored solid with a melting point of 144°C. It thermally decomposes at temperatures greater than 200°C. It has a low vapor pressure ( $1.5 \times 10^{-9}$  millimeters [mm] Hg at 20°C). Imidacloprid exhibits relatively high water solubility (610 milligrams per liter [mg/L]) and an octanol-water partition coefficient (log Kow) of 0.57, so it partitions only weakly to sediment. In sterile aquatic systems imidacloprid was found to be stable at all pH values tested. In contrast, it is highly photoreactive in aqueous solution with a half-life of 4.2 hours (h) determined under conditions of full sunlight at latitude = 50° N. The short half-life was corroborated in a quantum yield experiment, which resulted in half-life predictions ranging from 0.2 to 6.7 days (d), depending on the season and latitude (0.4 and 0.28 d between April and June, 3.1 d in November, and 6.73 d in December at 50° latitude and 10° longitude) (European Food Safety Authority (EFSA) 2006).

#### ***Fate and behavior in water***

From the degradation products identified in aerobic water sediment studies, a degradation pathway has been proposed as shown on Figure 2. The compounds marked with an asterisk were found only in systems exposed to light. Under anaerobic conditions, NTN33893-desnitro occurs as a major degradation product.

Three major photo-degradation products: NTN33893-desnitro (M09), NTN33893-desnitro-olefine (M23), and NTN33893-urea(M12), as well as five minor ones: NTN33893-AMCP (M16), NTN33893-formyl-AMCP (M40), chloronicotinic acid (M14), NTN33893-dihydroxy-guanidine (M17), and NTN33893-ring-open-guanidine (M10), were identified as photo-transformation products of imidacloprid Figure 3. The reaction course to chloronicotinic acid proceeds from the parent by stepwise photo-degradation with oxygen. No intermediates from this chain of reactions could be detected.

The degradation and partitioning behavior of imidacloprid in the dark was studied in three natural systems of water and sediment. Imidacloprid disappears slowly from the water phases of water/sediment systems and is adsorbed to the



sediment, which can be explained by the moderate Koc values of the compound. In the sediments of three different systems maximum values of total applied radioactivity (TAR) of 10.3 percent, 23.5 percent, and 32 percent are reached after 60, 7, and 14 days, respectively.

Within the sediment, imidacloprid is degraded to NTN33893-desnitro (M09) and other products to a minor extent. M09 reached maximum concentration in the sediment with 6.3 percent TAR after 92 days. Bound residues were found between 8.2 and 17.4 percent at study end (30 or 92 days). The calculated disappearance half lives (DT50 values) of imidacloprid for the total systems are between 32 and 142 days.

Under more natural conditions, where sunlight is allowed to reach water-sediment systems, the half-life ranged from 4 to 20 days. One test using only pond water in the dark resulted in a half-life of 331 days. While the conditions of this test make it irrelevant to a real-world exposure analysis, the study is useful to demonstrate the strong influence of sunlight on the degradation rate of imidacloprid in aquatic systems. The only major (>10 percent) degradation product in dark aquatic systems was NTN33893-desnitro (M09); while in illuminated aquatic systems, NTN33893-desnitro (M09), NTN33893-urea (M12), and 6-chloro nicotinic acid (M14) were all formed as major degradation products. Not surprisingly, the same primary compounds found in the illuminated aquatic systems were also observed in the aqueous photolysis study.

The degradation of imidacloprid in anaerobic systems was confirmed in two studies performed in the dark. At 20°C, imidacloprid had a half-life of 36 days, with NTN33893-desnitro formed as the only major product. Under anaerobic conditions at 5°C, the reaction rate was slower (half-life of 95 days) with the same major degradate observed. Three outdoor pond studies were conducted and offer the opportunity to assess the "real world" dissipation of imidacloprid in aquatic systems. One pond study conducted in Texas and two pond studies conducted in Germany gave evidence of a rapid dissipation. Half-lives for the aqueous phase and the total system were estimated to be 7-10 days and 10-20 days for the two studies conducted in Germany (European Food Safety Authority [EFSA] 2006).

### **1.3.2 Imidacloprid Toxicity**

Imidacloprid is a systemic insecticide of the chemical class of chloronicotinyls/neonicotinoids. The compound acts on the nicotinergergic acetylcholine receptors (nAChR) in the nervous system of insects, and is therefore also effective on pests resistant to acetyl cholinesterase inhibitors.



Fish, amphibians, and aquatic algae are less sensitive to imidacloprid than certain aquatic invertebrates in terms of survival and growth. Among aquatic invertebrates, arthropods such as chironomid and mysid species are extremely sensitive to imidacloprid exposure, with observed adverse effects on survival, growth, and reproductive success.

### ***Fish***

The acute and chronic toxicity of imidacloprid to fish has been studied in standard laboratory species. A summary of the available studies is presented below. For freshwater species, static 96-hour acute LC50 values ranged from greater than 105 mg a.i./L for bluegill (Bowman and Bucksath 1990) to 211 mg a.i./L for rainbow trout (Grau 1988). A test with a saltwater species, sheepshead minnow, yielded a 96-hour acute LC50 value of 161 mg a.i./L (Ward 1990). Using the standard classification scheme proposed by the US Environmental Protection Agency/Environmental Fate and Effects Division (EPA/EFED 2001), imidacloprid would be classified as practically nontoxic to fish.

A 98-day flow-through early life stage test was conducted with rainbow trout in response to EPA's requirements for testing as part of the pesticide registration process (Cohle and Bucksath 1991; Gagliano 1992). No statistically significant or biologically important effects of imidacloprid exposure were observed with respect to egg viability, hatch, survival or behavioral variables. The most sensitive endpoint was a significant reduction in body length at 36 and 60 days post-hatch. The No Observable Apparent Effects Concentration (NOAEC) for this endpoint was 9.8 mg/L. Based on a re-analysis (Gagliano 1992) of the Cohle and Bucksath (1991) data for day-36 post-hatch body length, this study yields an NOAEC of 1.2 mg a.i./L and a Lowest Observable Apparent Effects Concentration (LOAEC) of 2.3 mg a.i./L. This effect, however, was not seen at 60 days post-hatch.

### ***Aquatic Invertebrates***

Standard laboratory studies on freshwater and saltwater species, as well as a microcosm study, have been conducted with technical grade imidacloprid. On the basis of both acute and chronic toxicity, crustaceans and aquatic insects are more sensitive to imidacloprid than fish. As expected for an insecticide, effects on insect larvae were more pronounced. The active substance imidacloprid is very toxic to chironomid larvae (LC50 at 24 h equals 55 µg a.i./L (SERA 2005). *Daphnia*, fish and algae were by at least three orders of magnitude less sensitive with the lowest EC50 obtained for *D. magna* at 85,000 µg a.i./L.



Endpoints (LC50 and LD50) of several aquatic invertebrates to imidacloprid were listed in a risk assessment conducted by SERA (2005). Freshwater amphipod crustaceans such as *Hyaella azteca*, the saltwater mysid, *Mysidopsis bahia*, and the fresh water insect midge, *Chironomus tentans*, are among the most susceptible species. In freshwater, the water flea, *Daphnia magna*, is relatively less susceptible, while in saltwater, the eastern oyster (*Crassostrea virginica*) was least susceptible. Acute toxicity values range from a 96-hour NOAEC of 0.000035 mg/L for *H. azteca* (England and Bucksath 1991), to a 96-hour NOAEC of 145 mg/L for eastern oyster (Wheat and Ward 1991). On the basis of longer-term studies designed to assess reproduction, growth and survival, *M. bahia* was the most sensitive species, with a 96-hour LC50 of 37 µg a.i. imidacloprid/L, and *D. magna* was the most tolerant species with a 21-day NOAEC for immobility of 1800 µg/L (Young and Blake 1990).

A 19-week microcosm study conducted as part of EPA's pesticide registration requirements for imidacloprid confirms the results of the above laboratory studies (Moring et al. 1992). Technical grade imidacloprid was applied to the surface of tanks containing a variety of phytoplankton, zooplankton, and macro-invertebrates at two week intervals, for a total of 4 applications. Concentrations ranging from 0 to 0.180 mg a.i./L were employed. Amphipods were determined to be the most sensitive species, with statistically significant impacts on abundance at some sampling intervals at the lowest concentration tested, yielding a (LOAEC of 0.002 mg a.i./L. Statistically significant decreases in populations of total macro-invertebrates as well as individual macro-invertebrate taxa (mayfly, midge, caddisfly, beetle, and amphipod) were most frequently observed (at different sampling endpoints) at imidacloprid concentrations ranging from 0.02 to 0.180 mg a.i./L. On the basis of these findings, the study authors recommended 0.006 mg a.i./L as a regulatory NOAEC for imidacloprid in aquatic systems. However, the results of previously discussed laboratory studies (Gagliano 1991; Ward 1991), as well as the results for amphipods at some sampling intervals in this study, suggest that the NOEL for mortality of mysids following chronic (21day) exposure is 0.6 µg a.i./L.

None of the imidacloprid metabolites tested (urea metabolite NTN 33519, 6-chloronicotinic acid, and NTN 33823) were as acutely toxic as technical grade imidacloprid in tests with the midge (*C. tentans*) or amphipod (*H. azteca*) (Bowers 1996a; Bowers and Lam 1998; Rooney and Bowers 1996; Dobbs and Frank 1996b). In tests with *M. bahia*, a formulation of imidacloprid, NTN 33893 240 FS, had the same order of acute toxicity as technical grade imidacloprid (Lintott 1992).

Dungeness crab are less sensitive to imidacloprid than shrimp and marine copepods. Temporary tetany has been observed at imidacloprid concentrations ranging from 500 to 5,000 µg/L (Patten 2011), though these effects are reversed upon termination of exposure. The LC50 (up to 108 hours after exposure) was found to be 6,500 µg/L for a 4-hour exposure. Again, any exposures to Dungeness crab will be at significantly lower concentrations and will be transitory as the incoming and outgoing tides first dilute and then wash away imidacloprid.

### **Aquatic Plants**

The acute toxicity of imidacloprid was tested on green algae as part of EPA's pesticide registration process (Heimbach 1989; Gagliano and Bowers 1991). These studies yielded NOAEC values for biomass and growth equivalent to the limits of the tests (i.e., 119 mg a.i./L for 5-day test with *Selanastrum capricornutum*; 10 mg a.i./L for *Scenedesmus subspicatus*).

A 4-day NOAEC of 6.69 mg a.i./L was determined for the diatom (*Navicula pelliculosa*) following exposure to a 21.6 percent imidacloprid formulation (Hall 1996).

Statistically significant decreases of cyanophyte populations (blue-green algae) were observed at concentrations of 0.020 mg/L and higher at some sampling points in the microcosm study of Moring et al. (1992). However, a laboratory study on blue-green algae in support of pesticide registration (Bowers 1996b) does not support the biological significance of the transient effects observed by Moring et al. (1992). On the basis of biomass and growth, Bowers (1996b) reports 4-day EC25 and EC50 values of 26.7 and 32.8 mg a.i./L, respectively, with a 4-day NOAEC of 24.9 mg a.i./L.

In summary, imidacloprid demonstrates a much lower toxicity in tests using aquatic plants than in aquatic invertebrates.

## **1.4 Previous Studies**

Large-scale trials using imidacloprid in Willapa Bay and Grays Harbor were conducted in 2008, 2009, 2010, and 2011 under Federal and State Experimental Use Permits. The 2008 and 2009 trials investigated the efficacy of a flowable formulation of imidacloprid, Nuprid® 2F (Nuprid; Nufarm Americas Inc., Burr Ridge, IL) and a granular material, Mallet® 0.5G (Mallet; Nufarm Americas Inc.), as determined by density of shrimp burrows.



In early 2010, both large (>1 acre) and small (<0.1 acre) plots were used for trial applications. Nuprid was consistently effective against burrowing shrimp regardless of substrate type or type and density of vegetative cover at an application rate of 2.0 lbs a.i./ac. Conversely, when Mallet was applied at 0.5 lb a.i./ac, it was found to be effective only on sandy sites with low percentages of vegetative cover.

Further studies in 2010 used application rates of 2.0 lb a.i./ac of Nuprid and 0.5 lb a.i./ac of Mallet on 10 acre plots. Data were collected for water column, porewater, and whole sediment concentrations of imidacloprid, as well as impacts of imidacloprid application to epibenthic and benthic invertebrates, salmonids, and green sturgeon.

Additional studies were conducted in 2011, using application rates of 0.5 lbs a.i./ac for both Nuprid and Mallet. Data were collected for efficacy against burrowing shrimp, impacts to Dungeness crab and epibenthic and benthic invertebrates, and concentrations of imidacloprid in eelgrass (*Zostera marina*) and in the water column and sediment porewater.

### **1.5 Data Gaps Proposed to be Addressed in 2012**

Results of previous experimental trials indicate that the efficacy of imidacloprid on burrowing shrimp is good under certain circumstances (sandy, un-vegetated sediment). Ecology recommends that additional trials using these methods be conducted in 2012. Studies undertaken in 2012 will evaluate the efficacy of imidacloprid as well as its impacts on biological resources such as epibenthic and benthic infauna, megafauna, and submerged aquatic vegetation both temporally and over differing substrate types. This work will inform determination of the size and extent of a SIZ, should one be required.

## **2.0 OBJECTIVES AND DESIGN OF THE INVESTIGATION**

The overall objectives of this study are to:

- Evaluate the efficacy of liquid (Nuprid) and granular (Mallet) formulations of imidacloprid to control burrowing shrimp in commercial shellfish beds;
- Determine if there are impacts on biological resources; and
- If impacts are observed, determine the spatial extent, duration, and magnitude of the sediment impact zone.

Specific study elements include:

- Continue assessments of efficacy of granular and flowable formulations of imidacloprid to suppress burrowing shrimp across a wide variety of conditions;
- Measure concentrations of imidacloprid in water following treatment and use the results to evaluate the extent of off-site movement;
- Measure concentrations of imidacloprid in eelgrass following treatment and assess the potential hazard to non-target organisms via consumption;
- Measure the concentrations and persistence of imidacloprid in sediment porewater and the potential for natural recovery of the SIZ, including field verification;
- Assess the magnitude, spatial extent, and duration of impacts to benthic and epibenthic invertebrates following imidacloprid treatment; and
- Combine these elements into a comprehensive description of the sediment impact zone related to imidacloprid applications in Willapa Bay.

Nuprid and Mallet imidacloprid formulations will be applied to test plots ranging in size from 5 to 10 acres. Surface water, sediment porewater, and eelgrass samples will be collected from within the test plots and from three transects between the test area and shore and two transects toward open water (Mallet) or along drainage streams, if present (Nuprid). An untreated control plot will also be sampled to aid in the efficacy determination and as a reference for evaluating impacts to other biological resources. The control and test plots will be located on intertidal lands owned by the shellfish growers and, if possible, on areas not previously treated with other pesticides.

The experimental design and sampling will allow sufficient comparisons of relevant parameters, descriptors, and other observations between or among:

- Treated and untreated plots; and
- Plots treated with granular imidacloprid at 0.5 lb a.i./ac and flowable imidacloprid at 0.5 lb a.i./ac.

These three treatments (Nuprid, Mallet, and untreated controls) will be applied according to a randomized design featuring blocks of similar substrate type



(grain size), cover and type of vegetation, density of burrowing shrimp, and bathymetry. Each treatment and control plot will be duplicated. These plots will be sampled for efficacy, water, sediment, infauna, and macrofauna (Tables 1a and 1b). Treatments within each block will be separated by at least 500 m to prevent potential cross-contamination by tidal water that might transport imidacloprid. All control plots will also be located at least 0.5 mile from carbaryl treatment areas. Plot sizes for Nuprid treatments will be 10 acres, Mallet plots will be 5–10 acres, and control plots will be 5–10 acres in size. Proposed treatment and control plot coordinates and locations are presented in Table 2a and Figure 4, respectively. Tables 1b and 2b identify back-up treatment plot locations in the event that one of the proposed locations is not suitable at the time of application. Specific details of the sampling design are presented in Section 3.0.

### **3.0 FIELD SAMPLING DESIGN AND METHODS**

Sample collection and analysis for water, porewater, vegetation, and infauna will follow an iterative approach and will be collected using the equipment and methods described in the following subsections. The numbers of samples presented in the associated tables are for one treatment plot only.

#### **3.1 Imidacloprid Application Methods and Parameters**

Treatments will be applied by hand, by using an ATV equipped with either a boom sprayer for Nuprid 2F or a granular spreader for Mallet 0.5G, or via aerial spraying (helicopter). Standard calibration protocol will be followed to assure rates are within 5 percent of target. Data collected prior to and at the time of application will include:

- Sediment type (grain size), type and density of vegetation, burrowing shrimp density, and management history;
- Equipment calibration protocols;
- Temperature of air, water and sediment at 4 cm depth;
- Wind speed and direction;
- Times to or from peak low tide and magnitude of peak low and high tides;
- Amount of water on plot at time of application;
- Length of time before flood tide;
- Direction of currents onto and off of treated area;



- Location of on-plot tidal drainage streams;
- Variation in plot elevation;
- Percent cloud cover and solar radiation (watts/m<sup>2</sup>);
- Precipitation, if any;
- Duration of application (start and stop time); and
- Treatment/application deviations.

### **3.2 Water Column Sampling**

#### **3.2.1 Sample Locations and Sampling Procedures**

Water column water samples will be collected for analysis of imidacloprid both within and adjacent to the treatment area according to the conceptual plan presented in Figures 5 and 6. Samples will be collected in the upside center of the treatment plot, as well as at 60, 120, 240, and 480 meters (m) from the plot edge on the upstream and downstream side of the plot. These distances may be altered, or some samples omitted, based on site specific conditions. For example if the shoreline is less than 480 upslope from the treatment area then the last sample would be taken at the shoreline regardless of distance. In all such cases, the site specific issue and the rationale for changing the sample distances will be documented. In addition, two samples will be taken 60 m outside the treatment plots in areas not expected to be impacted by imidacloprid (Figures 5 and 6). Flow direction will be determined prior to sample collection by using orange peels, wood chips or dyes approved for water use. Transect locations will be selected to capture the maximum amount of water flow that has traversed over the treated site. Therefore, transects will likely not be straight lines radiating out as shown in the figures, but will be chosen based on actual tidal flow across the treatment plots. One pre-treatment sample will be taken within each treatment and control plot.

For the Nuprid treatment, water column samples will be collected on the first incoming tide following treatment (approximately 2 hours after application). Water will be collected along three transects on the upstream side of the plot immediately following treatment (Figure 5). Inundation water for the first incoming tide will be collected by burying the sample jar upright in the sediment with the mouth of the jar (4-oz. amber glass) approximately five centimeters above the sediment surface. After the sample jars fill, they will be quickly capped and removed from the sediment, and placed on ice in a cooler. If drainage streams exist downslope from the Nuprid treatment plots, samples will be collected within these streams at distances of 60, 120, 240, and 480 m from



the treatment plot, or until the drainage stream merges with a main channel or the advancing edge of the incoming tide. Sample points will follow the path of the drainage stream, not necessarily a straight transect. These samples will be collected as close to the sediment-water interface as possible without disturbing the sediment during sampling (~ 5 to 10 cm). One water sample will also be taken at the intersection of the drainage stream and the main channel or edge of the incoming tide. One sample will be taken in the main channel, 10 m downstream of the intersection with any sampled drainage stream, if site topography allows. These samples will be taken approximately 1 m below the water surface or mid-water column if total depth is less than 1 m. The samples will be collected either by hand or by using a pole and aperture system that can hold a sample jar closed at depth. Once the jar is at the appropriate depth, the aperture would be opened and the water column sampled. In addition, two samples will be taken 60 m outside the treatment plot in areas not expected to be impacted by imidacloprid (Figure 6).

For the Mallet treatment plot, imidacloprid will be applied in 0.5 to 3 feet of water during the outgoing tide. To capture movement of imidacloprid in the ebb tidal water, we will sample immediately following the complete application of the granular product to the site. These samples will be collected on the downstream side of the treatment plot (as the tide is ebbing off the treated plot) as shown in Figure 6. The samples will be collected in the middle of the water column either by hand or by using a pole and aperture system that can hold a sample jar closed at depth. Once the jar is at the appropriate depth, the aperture would be opened and the water column sampled. Samples will also be collected in three upstream transects on the first incoming tide after application (approximately 2 hours after treatment), using a similar protocol for sediment-water interface sampling as for Nuprid. In addition, two samples will be taken 60 m outside the treatment plot in areas not expected to be impacted by imidacloprid (Figure 5).

The control plot will have one sample taken from the upper middle of the plot, both pre-treatment and approximately 2 hours after treatment, on the first incoming tide.

After collection, water column samples will be placed in a cooler with ice and transported to the laboratory under chain of custody. Sample container, preservation, and holding time specifications are presented in Table 3.



### **3.2.2 Sampling Analysis Sequence and Decision Path**

Samples will be analyzed on an iterative basis. The analytical decision logic is summarized in Table 4. As discussed in the previous section, water column samples will be collected pre-treatment and on the first flood tide after application. All pre-treatment and on-plot samples will be analyzed within appropriate holding times (Table 3). The 60 m samples will be analyzed immediately, on a 2 day turnaround time. If imidacloprid concentrations are less than a 3.7 µg/L screening level in the 60 m samples, samples collected at subsequent sampling distances will not be analyzed. If imidacloprid is detected at concentrations greater than the 3.7 µg/L screening level, subsequent sample points along that specific transect will be analyzed.

The 3.7 µg/L screening level for surface water is a conservative concentration based upon EPA guidance (EPA 1985) that recommends an operational water quality criterion equal to one-tenth the LC50 for the most sensitive organism, in this case mysid shrimp with an LC50 of 37 µg/L. This LC50 value is based on a 96-hour exposure test using a constant concentration of imidacloprid. The epibenthic and benthic organisms in the imidacloprid treatments outlined in this SAP, by contrast, will be exposed to water-based concentrations of imidacloprid for at most a few hours as the incoming and outgoing tides first dilute and then wash away imidacloprid. Thus, use of 0.1 times a 96-hour LC50 for the most sensitive taxon tested is a very conservative screening level since the toxicology data for water based toxicity would have supported using a higher screening level.

## **3.3 Sediment Porewater Sampling**

### **3.3.1 Sample Locations and Sampling Procedures**

Sediment samples will be collected for porewater extraction and analysis both within and adjacent to the treatment area according to the conceptual plan presented in Figures 7 and 8. Samples will be collected in the center of the treatment plot and halfway between the center of the plot and the outer edges in all four directions (Figures 7 and 8). Samples outside the treatment plots will be collected from three transects on the upstream (nearshore) side of the treatment plot at 60, 120, 240, and 480 m from the plot edge. These distances may be altered, or some samples omitted, based on site-specific conditions. For example, if the shoreline is less than 480 upslope from the treatment area, then the last sample would be taken at the shoreline regardless of distance. In all such cases, the site-specific issue and the rationale for changing the sample distances will be documented.



Samples will be collected from within the drainage stream(s) on the downstream side of the Nuprid treatment plot (Figure 8) and from two transects on the downstream side of the Mallet treatment plot (Figure 7). In addition, two samples will be taken 60 m outside the treatment plots in areas not expected to be impacted by imidacloprid (Figures 7 and 8).

Sediment samples will be collected at low tide on days 1, 14, 28, and 56 after application. One pre-treatment sample will also be taken from the center of each treatment plot. Sediment cores collected after day 1 will be rotated clockwise in cardinal directions and offset 1 meter from the original sample point. For example, the day 14 sample will be collected 1 meter east of the day 1 sample, the day 28 sample will be collected one meter south of the day 1 sample, etc.

The control plots will have one sample taken from the center of the plot on each sampling day (Table 5).

Sediment samples will be collected using a coring device designed to collect a sample 10 centimeters (cm) in depth. The coring device is a modified semi-transparent, 500-ml HDPE bottle (7-cm diameter) with the bottom removed and a hole drilled into the top shoulder of the bottle to create vacuum and allow the cores to be removed.

Lightweight, disposable plastic coring devices and sample containers are preferable to a stainless steel corer and glass containers to reduce weight and to reduce the risk of cross-contamination among samples. Test sites are remote and accessible only by walking 1/2 to 2 miles in soft mud and the tidal window available for sampling lasts a maximum of 6 hours. Therefore, 1-L HDPE bottles will be used for transporting sediment porewater samples. In addition, the octanol-water partition coefficient for imidacloprid is low ( $\log K_{ow} = 0.57$ ) so only minimal partitioning to organics would be predicted.

Two cores will be collected at each sampling location to ensure enough sediment is collected to extract sufficient volumes of porewater. Samples will be placed in 1-L HDPE bottles and in a cooler on ice, then transported to the laboratory under chain of custody. Sediment porewater samples will not be frozen prior to extraction of porewater. All samples will be extracted within the 7-day holding time and the extracted porewater stored at 4°C until analyzed. Sample container, preservation, and holding time specifications are presented in Table 3.

### 3.3.2 Sample Analysis Sequence and Decision Path

Samples will be analyzed on an iterative basis. The analytical decision logic is summarized in Table 5. As discussed in the previous section, sediment samples will be collected pre-treatment and at 1, 14, 28, and 56 days after application. If imidacloprid concentrations are less than a 0.6 µg/L screening level in porewater samples collected from within the treatment areas, samples collected at later dates will not be analyzed. For the control sites, if imidacloprid concentrations are less than the 0.6 µg/L screening level, samples subsequently collected from that location will not be analyzed.

Samples outside the treatment area boundaries will be analyzed using a time, distance, and concentration-based iterative process. If imidacloprid porewater concentrations are less than 0.6 µg/L, samples collected at later dates from a given location will not be analyzed. In addition, samples collected more distant from the location will also not be analyzed. For example, if the imidacloprid concentration in the day 14 porewater sample collected 60 m from the edge of the treatment area is less than 0.6 µg/L, the day 28 and day 56 samples collected from this same location would not be analyzed. In addition, the day 14, 28, and 56 samples collected 120, 240, and 480 m from the test plot boundary on this transect would not be analyzed. However, if imidacloprid is still greater than 0.6 µg/L after 56 days, sampling will continue monthly until all samples have concentrations less than 0.6 µg/L.

The 0.6 µg/L screening level for sediment porewater is a conservative concentration based upon chronic effects NOEC in 21-day toxicity studies (Ward 1991), since sediment imidacloprid concentrations are at least somewhat persistent, and therefore can produce toxicity from chronic exposure. Mysid shrimp toxicity studies submitted as part of the EPA pesticide registration process (Section 3.1) demonstrated that this taxon was among the most susceptible of any species tested. Therefore, although mysid shrimp live within the water column rather than the sediment, a screening level equal to the NOEC concentration for this species (0.6 µg/L) was selected as the screening level. Based on toxicity studies for benthic arthropods that actually live in sediments, a NOEC screening concentration up to 6 µg/L could be supported, indicated that the screening level proposed here is as much as an order of magnitude more conservative than actual mortality risk from the planned imidacloprid treatments.



### 3.4 Vegetation Sampling

#### 3.4.1 Sample Locations and Sampling Procedures

Eelgrass (*Zostera marina* or *Zostera japonica*) will be collected both within the treatment area and outside the treatment area according to the conceptual plan presented in Figure 9.

Eelgrass (*Z. marina* or *Z. japonica*) will be sampled from two on-plot sampling points where density is high enough to facilitate sampling. *Z. marina* will be preferentially collected; however, if it is not present, or present in insufficient quantities, *Z. japonica* will be collected instead. Eelgrass will be sampled pre-treatment and at intervals of 1, 14, and 28 days post treatment from two locations within each treatment plot, six locations outside each treatment plot (60 m and 120 m) on the high-water side, and in the control plot. Average biomass of eelgrass tissue is approximately 245 grams dry weight per square meter (gDW m<sup>2</sup>; Olesen and Sand-Jensen 1994) and wet weight is approximately 4–5 times that of dry weight (J. Stutes, personal communication). Therefore, the equivalent of approximately 0.25 m<sup>2</sup> of eelgrass bed will be needed for each sample, or slightly more if the eelgrass being sampled is sparse.

Eelgrass will be sampled by hand to carefully remove the aboveground parts of the plant. Eelgrass samples will be placed in 1 gallon self-sealing plastic bags and placed on ice in a dark cooler. Within 30 minutes of collection the sample will be moved approximately 1000 m outside the treatment zone and triple rinsed with 2 L of clean bay water to remove any surface sediment and residue. The sample will then be placed in clean 1-gallon self-sealing plastic bags, placed in a cooler on ice, and transported to the laboratory under chain of custody. Sample container, preservation, and holding time specifications are presented in Table 3.

#### 3.4.2 Sample Analysis Sequence and Decision Path

Samples will be analyzed on an iterative basis. The analytical decision logic is summarized in Table 6. As discussed in the previous section, vegetation samples will be collected pre-treatment and at low tide after application. All samples inside of the treatment plots will be analyzed until imidacloprid concentrations in the eelgrass are less than 10 µg/L. The screening level of 10 µg/L is equal to the quantitation limit for imidacloprid in this sample type (Steve Thun, Pacific Agricultural Laboratory, personal communication). Samples outside the treatment plots will be analyzed iteratively based on concentrations of imidacloprid found in the water column. If analysis of water from any 60- or 120-m sample location is greater than 10 µg/L during the first flood tide, then

vegetation from those sample locations will be analyzed. Vegetation samples collected on days 14 and 28 will only be analyzed when eelgrass from the previous sampling event had detectable imidacloprid concentrations. If imidacloprid is still detectable after 28 days, sampling will continue until all samples have concentrations less than 10 µg/L.

Past sampling of eelgrass has failed to detect imidacloprid in all but one collected sample. If this pattern continues in 2012, then the iterative procedure would likely exclude analysis of most collected eelgrass samples. However, in 2012 we will also be testing eelgrass samples for two degradation products of imidacloprid (see section 3.8 below). Should either of these products be detected (alone or in combination) at concentrations above 10 µg/L, then these detections will be considered the same as detection of imidacloprid with respect to triggering analysis of eelgrass samples under the iterative procedure.

### **3.5 Epibenthic and Benthic Invertebrate Sampling**

#### **3.5.1 Sample Locations and Sampling Procedures**

Epibenthic and benthic samples will be collected both within and adjacent to the treatment area according to the conceptual plan presented in Figure 10.

Epibenthic (crustaceans) and benthic invertebrates will be sampled adjacent to the selected sediment sampling stations inside and outside the treatment plots and in the control plot. Epibenthic and benthic invertebrates will be sampled prior to the application of imidacloprid and at 14 and 28 days post-treatment. The 14-day time period is meant to allow invertebrates killed by imidacloprid exposure to begin decomposition so that they can be differentiated from invertebrates alive at the time of sample collection that were subsequently killed by exposure to formalin.

Four replicate core samples will be collected at each of five on-plot sample stations for a total of 20 on-plot replicates on both the treatment and control plots (Figure 10). The location of the off-plot stations will be decided based on the results of the water quality sampling conducted on the day of treatment. Specifically, the results of the water sampling will be available prior to the 14-day post-treatment invertebrate sampling, and so the location of the 3.2 µg/L screening level for water concentrations of imidacloprid will be known. Based on this location, invertebrate samples will be taken upslope of the treatment area at site-specific distances reflective of the location of the boundary and site conditions. For example, if water samples with imidacloprid concentrations of 3.2 µg/L screening level were observed 120 m upslope from the treatment area,



we could collect two off-plot samples at 60 m and two at 120 m from the treatment plot boundaries, on the high-water side. Were the boundary to be at 300 m, then samples could be taken at 100, 200, and 300 m. The specific sampling locations, and the rationale for selecting those locations, will be documented for all sampling events and sites.

Additionally, four replicate cores samples will be taken at each of four control plot locations on each of the dates that treatment plot samples are taken.

Invertebrates will be sampled using a 10.2-cm internal diameter corer to a depth of 10 cm (Photograph 1). Cores will immediately be sieved through a 0.5-mm mesh sieve (Photograph 2) using salt water, then stored in a 10 percent buffered formalin solution for 2–4 weeks. Sample jars will be labeled according to specific sample station, replicate number, and sample date on the inside and outside. Samples will then be re-sieved through a 100- $\mu$ m mesh sieve using freshwater, transferred to 70 percent isopropyl alcohol, stained with rose Bengal, and stored at room temperature until identified and counted.

### **3.5.2 Taxonomic Identification and Analysis**

Epibenthic and benthic invertebrate sample identification will be conducted by Ruff Systematics and Pacific Shellfish Institute (PSI) staff. All Crustaceans, Mollusks, and Polychaetes will be identified to the lowest taxonomic level possible.

The primary metric of comparison for treatment effect will be by direct comparison of absolute abundance, taxonomic richness, and Shannon-Wiener diversity of organisms within each of Class Crustacea, Class Polychaeta, and Phylum Mollusca on beds treated with imidacloprid to those of untreated beds (reference or check beds). An effect will be established when abundance or richness on a treated site is <50 percent of the mean values on the untreated bed as determined by one-tailed t-test ( $\alpha=0.05$ ). Comparisons will be made at each sample interval so the duration of any impact can also be determined.

An additional analysis will feature comparisons of the change in the proportions of the primary descriptors on the treated bed between sample intervals. If the proportions do not change substantially after treatment, impact can be assumed to be minimal. If the proportions decline substantially after treatment, the impact can be assumed to be correspondingly greater. Note that a proportion of <33 percent is equivalent to the ratio of <50 percent that was used in the primary comparison, as described above. Change in the proportions of abundance, richness, and diversity provide a better assessment than change in

the ratios between treated and untreated plots, as the latter sometimes involves dividing by zero, resulting in missing values and bias of results. Proportions will be arcsine-transformed prior to statistical analyses (t-test or one way-analysis of variance [ $\alpha=0.05$ ]).

### 3.5.3 Sample Analysis Sequence and Decision Path

Epibenthic and benthic invertebrate samples taken within the treatment and control plots will be analyzed on an iterative basis (Table 7). Three of the four replicate samples taken from each of the five stations within the treatment and control plots will be analyzed for all sample times. The remaining replicates at these sample stations will be re-sieved and placed in isopropyl alcohol, but will not be further processed or analyzed until the results of the initial 15 replicates are examined using power analysis. Regardless of whether 3 or 4 replicates are ultimately analyzed, all five sample locations inside of the treatment and control plots will be analyzed until porewater imidacloprid concentrations in the treatment plots are less than 0.6 µg/L. If they remain above 0.6 µg/L after 28 days, then additional invertebrate samples at later dates will be taken.

As noted above, epibenthic and benthic invertebrates will be sampled outside of the treatment plots based on the boundary of the water screening level of 3.2 µg/L, and site-specific conditions. By contrast, the determination as to which of these samples will be analyzed will be determined based on the results of sediment porewater samples analyzed for imidacloprid concentrations. For a given date, if analysis of porewater from any sample location outside the treatment plot is greater than 0.6 µg/L, then epibenthic and benthic invertebrates from those sample locations will be analyzed. If imidacloprid concentrations in porewater are still greater than 0.6 µg/L after 28 days, sampling will continue until all samples are less than 0.6 µg/L.

For each sample taken outside the treatment plots, we will initially analyze four of the eight replicates taken from that location. Subsequent analysis of additional replicates will only be undertaken if results of a power analysis indicate that they need to be analyzed.

The exception to analysis of samples from outside the treatment plots would occur when both of the following are true: (1) analysis of invertebrate samples from the control location and the treatment plot for a given date failed to show a decrease in any tested taxon >50 percent, and (2) the water samples taken on the day of application did not detect any off-plot location with higher concentrations than those measured on the treatment plots. Under this exception, no invertebrate samples from outside the treatment plot would be



analyzed because samples analyzed from areas of higher imidacloprid exposure (i.e., on the treatment plot itself) will already have demonstrated that treatment effects, as defined in this SAP, are not present.

### **3.6 Megafauna Sampling**

Megafauna (Dungeness crab and fish) will be counted 24 hours after treatment at low tide, along 3- to 7-m wide transects that cross and extend 50 m on both sides of each plot. Species, size, incidence of tetany, and cause of death will be recorded for every individual sighted.

### **3.7 Efficacy Sampling**

Burrowing shrimp and polychaete burrows will be counted on the day preceding treatment and at 14 and 60 days after treatment on both the treated and control plots. Ten 0.25-m<sup>2</sup> counts will be made at 10 predetermined, marked locations within each site. Mean number of burrows per m<sup>2</sup> will be compared between treated and control plots (t-test,  $\alpha = 0.05$ ) and among sample intervals (ANOVA,  $\alpha = 0.05$ ). Additional before and after counts will be made at distances of 30 m and 60 m immediately in each cardinal direction from the treated plot.

### **3.8 Degradation Products Analysis**

EPA, in comments on an earlier version of the SAP, requested that two derivatives of imidacloprid, imidacloprid olefin [1-(6-chloro-3-pyridylmethyl)-N-nitro-1,3-dihydro-imidazol-2-ylideneamine] and 5-hydroxy imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-5-ol] be analyzed, given some literature indicating that these degradation products of imidacloprid themselves can exert biological toxicity. It has been difficult to find a source for analytical standards for these degradation products, effectively preventing their analysis as part of the SAP. One lab (ChemServe) has indicated that they may be able to provide such standards. These two derivatives of imidacloprid will be analyzed in treatment area porewater and vegetation samples submitted for imidacloprid analysis as long as the analytical standards can be obtained in a timely manner. Because such standards are not commercially available, ChemServe will have to conduct specialty synthesis, purification, and verification of compound purity.

Treatment area pore water and vegetation samples taken on day 14 and subsequent dates would be analyzed on an iterative basis for these derivatives. Any samples from earlier dates, for example the water samples collected several hours after treatment, will not be analyzed for degradation products of imidacloprid because too little time for degradation will have occurred. The

iterative process for these samples will be the same as for the imidacloprid analysis of porewater and vegetation (see Sections 3.3.2 and 3.4.2); i.e., if the concentration of imidacloprid and breakdown products is less than the screening level, subsequent samples will not be analyzed. For this determination, the concentrations of imidacloprid and the derivatives are assumed to be linearly additive. Thus any combination of concentrations for the 3 chemicals that sums to or greater than the screening levels will trigger the analysis of samples from that location.

### **3.9 Decontamination Procedures**

To prevent sample cross-contamination, all non-dedicated sampling and processing equipment (e.g., stainless steel spoons and bowls) used during sediment and eelgrass sampling and processing will be thoroughly decontaminated before use following this procedure:

- Rinse with water and wash with scrub brush until free of sediment;
- Wash with Liquinox detergent and tap water transported to the site;
- Rinse with tap water; and
- Rinse three times with distilled or deionized water.

All personnel engaged in sample collection and handling will wear disposable nitrile gloves. Gloves will be disposed of between water, sediment, and eelgrass samples to prevent cross-contamination.

### **3.10 Sample Containers and Labels**

Sample container requirements vary according to analyte and sample matrix. Pre-cleaned sample containers will be obtained from the analytical laboratory. Sample containers shall be cleaned following the requirements described in Specifications and Guidance for Contaminant-Free Sample Containers (EPA 1992, OSWER Directive 92.0-05a). Required storage temperatures and holding times are summarized in Table 3.

### **3.11 Field Documentation Procedures**

Field notes will be maintained during sampling and processing operations. The following will be included in the field notes:



- Names of the field sampling crew, including person(s) collecting and logging the samples;
- Weather conditions;
- GPS coordinates of each sampling location;
- Date and time of collection of each sample; and
- Any deviation from the approved sampling plan.

## **4.0 SAMPLE HANDLING PROCEDURES**

### **4.1 Sample Storage Requirements**

#### **4.1.1 Chemical and Physical Analyses**

Samples will be preserved according to the requirements of the specific analytical methods to be employed, and all samples will be extracted and analyzed within method-specified holding times. Sample storage temperatures and holding times are summarized in Table 3.

#### **4.1.2 Epibenthic and Benthic Invertebrate Samples**

Epibenthic and benthic invertebrate samples will be sieved through a 0.5-mm mesh sieve using salt water, then stored in a 10 percent buffered formalin solution for 2–4 weeks. They will then be re-sieved through a 100- $\mu$ m mesh sieve using freshwater, transferred to 70 percent isopropyl alcohol, stained with rose Bengal, and stored at room temperature until identified and counted.

### **4.2 Chain of Custody Procedures**

Chain of custody forms will be used to document the collection, custody, and transfer of samples from their initial collection location to the laboratory and their ultimate use and disposal. Entries for each sample will be made on the custody form immediately after each sample is collected.

Sample custody procedures will be followed to provide a documented record that can be used to follow possession and handling of a sample from collection through analysis. A sample is considered to be in custody if it meets at least one of the following conditions:

- The sample is in someone's physical possession or view;

- The sample is secured to prevent tampering (i.e., custody seals); and/or
- The sample is locked or secured in an area restricted to authorized personnel.

A chain of custody form will be completed in the field as samples are packaged. At a minimum, the information on the custody form shall include the sample number, date and time of sample collection, sampler, analyses, and number of containers. One copy of the custody form will be placed in the cooler prior to sealing for delivery to the laboratory with the respective samples. A second copy will be retained and placed in the project files after review by the Project Chemist. Custody seals will be placed on each cooler or package containing samples so that the package cannot be opened without breaking the seals.

#### ***4.3 Delivery of Samples to Analytical Laboratory***

After sample containers have been filled, they will be packed on ice in coolers. The coolers will be transferred to Pacific Agricultural Laboratory for chemical analysis. Specific procedures are as follows:

- Samples will be packaged and shipped in accordance with US Department of Transportation regulations as specified in 49 CFR 173.6 and 49 CFR 173.24.
- Individual sample containers will be packed to prevent breakage.
- The coolers will be clearly labeled with sufficient information (name of project, time and date container was sealed, person sealing the cooler, and the return address) to enable positive identification.
- A sealed envelope containing custody forms will be enclosed in a plastic bag and taped to the inside lid of the cooler.
- Signed and dated custody seals will be placed on all coolers prior to shipping.
- Samples will either be shipped by overnight courier or will be hand delivered to the laboratory.
- Upon transfer of sample possession to the testing laboratories, the custody form will be signed by the persons transferring custody of the coolers. Upon receipt of samples at the laboratory, the shipping container custody seal will be broken and the custodian receiving laboratory samples will compare



samples to information on the chain of custody form and record the condition of the samples received.

## **5.0 LABORATORY ANALYTICAL METHODS**

Samples will be analyzed according to EPA methods as described in Test Methods for Evaluating Solid Waste; Physical/Chemical Methods, SW-846 (EPA 1986 and updates) and the Puget Sound Estuary Program Protocols (PSEP 1991 and updates), as referenced in Ecology's Sediment Sampling and Analysis Plan Appendix (SAPA; Ecology 2008). Sample methods, preparation, analyses, and practical quantitation limits are presented in Table 8.

In all cases, to avoid potential problems and leave open the option for retesting, sediments, tissue, and sample extracts will be kept under proper storage conditions until the chemistry data are deemed acceptable.

### **5.1 Chemical Analysis**

Chemical analysis will be performed for imidacloprid by high-performance liquid chromatography with thermospray tandem mass spectrometry (HPLC/MS/MS) using EPA Method 8321B. Two derivatives of Imidacloprid will also be analyzed with thermospray tandem mass spectrometry (HPLC/MS/MS) using EPA Method 8321B, if analytical standards can be obtained. These two derivatives are imidacloprid olefin [1-(6-chloro-3-pyridylmethyl)-N-nitro-1,3-dihydro-imidazol-2-ylideneamine] and 5-hydroxy imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-5-ol]. Laboratory-specific calibration and testing has not yet been completed for these derivatives (Steve Thun, Pacific Agricultural Laboratory, personal communication), but should be available prior to sample analysis.

#### **5.1.1 Sediment Porewater Extraction**

Porewater will be extracted from sediment samples by vacuum filtration. Approximately 400 grams of each sediment sample will be placed in a disposable, sterile, 500-ml Millipore Steritop® 0.22 micron filtration unit. Vacuum will be applied and the porewater extracted from the sample and collected into a clean amber glass bottle. The process will be repeated until a minimum volume of 50 mL of porewater is extracted. Extracted samples will either be analyzed immediately, or stored at 4°C until analysis to prevent exceedance of applicable holding times.

### **5.1.2 Laboratory Sample Extraction and Preparation**

Sample extraction and preparation methods are summarized in Table 6. Surface water and porewater samples will be extracted by solid phase extraction (SPE) using EPA Method 3535A. Plant tissue will be extracted by Association of Official Analytical Chemists (AOAC) Method 2007.01, Quechers extraction or FDA PAM 1302 (HPLC-MS).

### **5.2 Epibenthic and Benthic Invertebrate Analysis**

Epibenthic and benthic invertebrate samples that have been stored in isopropyl alcohol will be separated from detritus and sorted into separate vials of polychaetes, mollusks, and crustaceans for delivery to taxonomic identifiers (Photographs 3 and 4). Specific sample location, replicate number, and date will be labeled on the inside and outside of each vial. The original sample jars, including detritus in isopropyl alcohol, will be conserved.

## **6.0 QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC) REQUIREMENTS**

### **6.1 QA/QC for Chemical Analysis**

The quality of analytical data generated is controlled by the frequency and type of internal QC checks developed for analysis type. The quality of laboratory measurements will be assessed by reviewing results for analysis of method blanks, matrix spikes, duplicate samples, laboratory control samples, surrogate compound recoveries, instrument calibrations, performance evaluation samples, interference checks, etc., as specified in the analytical methods to be used. The following general procedures will be followed for all laboratory analyses:

- Laboratory blank measurements at a minimum frequency of 5 percent or one per batch of 20 samples or fewer for each matrix;
- Matrix spike (MS) and matrix spike duplicate (MSD) analysis, for organic analyses, to assess accuracy and precision at a minimum frequency of 5 percent or one per batch of 20 samples or fewer for each matrix;
- Analysis of surrogate compounds, for all organic analyses, to assess accuracy; and



- Laboratory control sample analysis to assess accuracy in the absence of any matrix effect at a minimum frequency of 5 percent or one per batch of 20 samples or fewer for each matrix.

Analytical method-specific requirements and criteria are summarized in Tables 9 and 10.

## **6.2 QA/QC for Biological Analysis**

Accurate and efficient taxonomic identification of the recovered animals requires careful handling of the samples during rescreening and sorting to minimize damage to the specimens. Sample sorting will be subjected to quality control checks. Taxonomic identifications will be checked against suitably verified reference collections or verified by independent taxonomists. Samples sent to other labs for independent verification need to follow the established laboratory COC procedures.

## **6.3 Data Quality Assurance Review Procedures**

An independent data quality review will be performed on the chemical analytical results provided by Pacific Agricultural Laboratory. This report will assess the adequacy of the reported quantitation limits in achieving the project screening levels; the precision, accuracy, representativeness, and completeness of the data; and the usability of the analytical data for project objectives. Exceedances of analytical control limits will be summarized and evaluated.

A data evaluation review will be performed on all results using QC summary sheet results provided by the laboratory for each data package. The data evaluation review is based on the Quality Control Requirements previously described and follows the format of the EPA National Functional Guidelines for Organic (EPA 2008) and Inorganic (EPA 2010) Data Review modified to include specific criteria of individual analytical methods. Raw data (instrument tuning, calibrations, chromatograms, spectra, instrument printouts, bench sheets, and laboratory worksheets) will be available for review if any problems or discrepancies are discovered during the routine evaluation or if Ecology desires a more comprehensive data validation be performed. The following is an outline of the data evaluation review format:

- Verify that sample numbers and analyses match the chain of custody request;
- Verify sample preservation and holding times;

- Verify that instrument tuning, calibration, and performance criteria were achieved;
- Verify that laboratory blanks were performed at the proper frequency and that no analytes were present in the blanks;
- Verify that field and laboratory duplicates, matrix spikes, and laboratory control samples were run at the proper frequency and that control limits were met;
- Verify that surrogate compound analyses have been performed and that results met the QC criteria; and
- Verify that required detection limits have been achieved.

Data qualifier flags, beyond any applied by the laboratory, will be added to sample results that fall outside the QC acceptance criteria. An explanation of data qualifiers to be applied during the review is provided below:

- **U.** The compound was analyzed for but was not detected. The associated numerical value is the sample reporting limit.
- **J.** The associated numerical value is an estimated quantity because QC criteria were slightly exceeded or because reported concentrations were less than the practical quantitation limit (lowest calibration standard).
- **UJ.** The compound was analyzed for, but not detected. The associated numerical value is an estimated reporting limit because QC criteria were not met.
- **R.** Data are not usable because of significant exceedance of QC criteria. The analyte may or may not be present; resampling and/or re-analysis are necessary for verification.

## **7.0 DATA ANALYSIS, RECORDKEEPING, AND REPORTING REQUIREMENTS**

### **7.1 Analysis of Chemistry Data**

Chemistry results will be compared to the following project screening levels:



- Surface water – 3.7 µg/L,
- Interstitial sediment porewater – 0.6 µg/L, and
- Eelgrass – 10 µg/kg (wet weight basis).

## **7.2 Analysis of Biological Test Data**

Epibenthic and benthic invertebrate sample identification will be conducted by Ruff Systematics and PSI staff. The primary metric of comparison for treatment effect will be by direct comparison of absolute abundance, taxonomic richness, and Shannon-Wiener diversity of organisms within each of Class Crustacea, Class Polychaeta, and Phylum Mollusca on beds treated with imidacloprid compared to that of untreated beds (reference or check beds). An effect will be established when abundance or richness on a treated site is <50 percent of the mean values on the untreated bed as determined by one-tailed t-test ( $\alpha=0.05$ ). Comparisons will be made at each sample interval so the duration of any impact can also be determined.

An additional analysis will feature comparisons of the change in the proportions of the primary descriptors on the treated bed between sample intervals. If the proportions do not change substantially after treatment, impact can be assumed to be minimal. If the proportions decline substantially after treatment, the impact can be assumed to be correspondingly greater. Note that a proportion of <33 percent is equivalent to the ratio of <50 percent that was used in the primary comparison, as described above. Change in the proportions of abundance, richness, and diversity provide a better assessment than change in the ratios between treated and untreated plots, as the latter sometimes involves dividing by zero, resulting in missing values and bias of results. Proportions will be arcsine transformed prior to statistical analyses (t-test or one way analysis of variance ( $\alpha=0.05$ )).

## **7.3 Recordkeeping Procedures**

Project records will be kept and maintained in accordance with SMS requirements for a minimum of 10 years following completion of issuance, modification, or renewal of applicable project permits, administrative order, certification, or project cleanup site delisting, whichever is greater. Records will include:

- This SAP and related quality assurance documentation;
- Field records identifying sampling dates, types, composites, locations, and depths;



- Lists of sampling personnel, equipment, methods, and procedures;
- Sediment analysis records (laboratory analytical documentation);
- Final report; and
- Any departures from SAP and quality assurance plans.

## **7.4 Reporting Procedures**

### **7.4.1 Physical and Chemical Analysis Laboratory Reports**

The laboratory data reports will consist of complete data packages that will contain complete documentation and all raw data to allow independent data reduction and verification of analytical results from laboratory bench sheets, instrument raw data outputs, and chromatograms. Each laboratory data report will include the following:

- Case narrative identifying the laboratory analytical batch number, matrix and number of samples included, analyses performed and analytical methods used, and description of any problems or exceedance of QC criteria and corrective action taken. The laboratory manager or their designee must sign the narrative.
- Copy of chain-of-custody forms for all samples included in the analytical batch.
- Tabulated sample analytical results with units, data qualifiers, percent solids, sample weight or volume, dilution factor, laboratory batch and sample number, field sample number, and dates sampled, received, extracted, and analyzed all clearly specified. Surrogate percent recoveries will be included for organic analyses.
- Surrogate spike recoveries will be reported in all organic reports where appropriate. The reports shall also specify the control limits for surrogate spike results, as well as the spiking concentration. Any out of control recoveries will be reported immediately to the Project QA Manager. Any out-of-control recoveries (as defined in the method) will result in the sample being rerun (both sets of data are to be reported).
- All calibration, quality control, and sample raw data, including chromatograms, quantitation reports, and other instrument output data.
- Blank summary results indicating samples associated with each blank.

- Matrix spike/matrix spike duplicates result summaries with calculated percent recovery and relative percent differences.
- Laboratory control sample results, when applicable, with calculated percent recovery.
- Electronically formatted data deliverable (diskette) results.

#### **7.4.2 Reports to Ecology**

A report will be prepared summarizing sampling procedures and laboratory testing results. The report will include a map with confirmed sampling locations, tabulated analytical testing data, and complete laboratory analytical documentation. At a minimum, the report will include the following sections

- Introduction/Purpose;
- Vicinity map;
- Summary of field sampling and laboratory procedures and any deviations from the SAP;
- Figure and table documenting sample locations and coordinates;
- Tabulated results of sediment chemistry;
- Data validation review and laboratory report sample summary and quality control results;
- Discussion and interpretation of results; and
- Conclusions.

### **8.0 SCHEDULE**

This study is tentatively planned to begin in early May or early June 2012. The dates for this study are dependent upon obtaining permits, weather, and availability of helicopter pilots, if necessary.

## 9.0 PROJECT PERSONNEL AND RESPONSIBILITIES

Key institutions staff members for this task order are listed below with their project functions.

- Washington State University Extension – Overall Scientific Management
  - Dr. Kim Patten, Director, Long Beach Research Center
  - Nick Haldeman, Technician, Long Beach Research Center
- University of Washington, School of Aquatic and Fishery Sciences – Lead in Sediment Porewater Studies
  - Dr. Chris Grue, USGS and Associate Professor and Leader, Washington Cooperative Fish and Wildlife Research Unit (WACFWRU)
  - Martin Grassley, Research Scientist
  - John Frew, PhD student
- Pacific Shellfish Institute – Lead in Invertebrate Studies
  - Kristin Rasmussen, Executive Director
  - Dr. Steven R. Booth, Senior Scientist
  - Andy Suhrbier, Senior Biologist
  - Mary Middleton, Senior Biologist
- Ruff Systematics – Assistance with Invertebrate Studies
  - R. Eugene Ruff
- Pacific Agricultural Laboratory – Analytical Laboratory for Sample Testing
  - Steve Thun, Director

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## TABLES



**Table 1a – Experimental Site Plan for 2012 Experimental Use Permit Application for Imidacloprid in Willapa Bay – Proposed Plots**

Location	Substrate	Average Burrow Density February 2012 (#/m <sup>2</sup> )	Vegetation	Proposed Treatment	Monitoring Plan
Stackpole (Leadbetter)	Sand	15	None	10 ac aerial applied Nuprid, 5 ac hand apply Mallet, adjacent control	Efficacy, water, sediment, infauna, macrofauna
Rhodesia Beach (Bay Center)	Sand	10	<i>Z. japonica</i> thin to moderate	10 ac ATV applied Nuprid, 5-10 ac ATV, boat, and/or hand apply Mallet, adjacent control	Efficacy, water, sediment, infauna, macrofauna

**Table 1b – Experimental Site Plan for 2012 Experimental Use Permit Application for Imidacloprid in Willapa Bay – Back-up Plots**

Location	Substrate	Average Burrow Density February 2012 (#/m <sup>2</sup> )	Vegetation	Proposed Treatment	Monitoring Plan
Palix (Bay Center)	Sand with some silt	20	<i>Z. marina</i> sparse	10 ac aerial applied Nuprid	Efficacy, water, macrofauna
Nachotta Spit (Nachotta)	Sand with some silt	25	None	10 ac hand applied Nuprid	Efficacy, water, macrofauna
Cedar River	Sand	10	<i>Z. marina</i> sparse	10 ac aerial applied Nuprid	Efficacy, water, macrofauna

**Table 2a - Proposed Treatment and Control Plot Location Coordinates**

Location	WGS 84 Decimal Degrees		WGS 84 Decimal Degrees	
	Treatment Sites		Control Site	
	Latitude	Longitude	Latitude	Longitude
Stackpole (Leadbetter)	46.620	-124.035	46.620	-124.043
Rhodesia Beach (Bay Center)	46.592	-123.845	46.588	-123.945

**Table 2b – Back-up Treatment and Control Plot Location Coordinates**

Location	WGS 84 Decimal Degrees		WGS 84 Decimal Degrees	
	Treatment Sites		Control Site	
	Latitude	Longitude	Latitude	Longitude
Palix (Bay Center)	46.633	-123.942	46.629	-123.943
Nachotta Spit (Nachotta)	46.493	-124.020	46.494	-123.029
Cedar River	46.712	-123.951	46.708	-123.943

**Table 3 – Sample Containers, Preservation, and Holding Times**

Sample Type	Container	Sample Preservation	Holding Time
Imidacloprid			
Sediment for porewater extraction	500 mL HDPE jar	Cool, 4° C, dark	7 days
- Extracted porewater	8 oz amber glass bottle	Cool, 4° C, dark	40 days <sup>a</sup>
Surface water	4 oz amber glass bottle	Cool, 4° C, dark	7 days <sup>a</sup>
Eelgrass	Self-sealing plastic bag	Cool, 4° C, dark	7 days
Post laboratory extraction (solvent exchanged)	Sealed laboratory vial	Cool, 4° C, dark	40 days

**Notes**

a – Pacific Agricultural Laboratory studies confirm imidacloprid is stable in seawater for more than 90 days when stored in the dark at 4°C.  
 Personal communication from Steve Thun, Laboratory Director, Pacific Agricultural Laboratory. February 17, 2012.



**Table 4 - Analytical Decision Logic for Water Column Samples**

<b>Water Column Samples - Mallet Treatment Area</b>			
	<b>Pre-treatment</b>	<b>2 Hours<sup>a</sup></b>	<b>Total</b>
Treatment Area	1	1	2
Off-plot Transect 1 (flood tide) <sup>b,c</sup>	0	4	4
Off-plot Transect 2 (flood tide) <sup>b,c</sup>	0	4	4
Off-plot Transect 3 (flood tide) <sup>b,c</sup>	0	4	4
Off-plot Transect 4 (ebb tide) <sup>b,c</sup>	0	4	4
Off-plot Transect 5 (ebb tide) <sup>b,c</sup>	0	4	4
Lateral Off-plot locations	0	2	2
<b>Total</b>	<b>1</b>	<b>23</b>	<b>24</b>
<b>Water Column Samples - Nuprid Treatment Area</b>			
	<b>Pre-treatment</b>	<b>2 Hours<sup>a</sup></b>	<b>Total</b>
Treatment Area	1	1	2
Off-plot Transect 1 (flood tide) <sup>b,c</sup>	0	4	4
Off-plot Transect 2 (flood tide) <sup>b,c</sup>	0	4	4
Off-plot Transect 3 (flood tide) <sup>b,c</sup>	0	4	4
Off-plot Drainage Stream <sup>b,c,d</sup>	0	4	4
Lateral Off-plot locations	0	2	2
<b>Total</b>	<b>1</b>	<b>19</b>	<b>20</b>
<b>Water Column Samples - Control Area</b>			
	<b>Pre-treatment</b>	<b>2 Hours<sup>a</sup></b>	<b>Total</b>
Treatment Area	1	1	2

**Notes:**

a - 2 hours is figurative, depending on timing of tides. Ebb tide Mallet transects will be sampled earlier than flood tide transects and any of the Nuprid sample points.

b - Samples will be collected along transects at distances of 60, 120, 240, and 480 meters from the boundary of the treatment area.

c - If imidacloprid water column concentrations are less than 3.7 µg/L in the 60 m samples, samples collected at subsequent sampling points will not be analyzed.

d - One main drainage stream is assumed, however if more than one exists in a plot, those streams will be sampled in the same manner.



**Table 5 - Analytical Decision Logic for Porewater Samples**

<b>Sediment Porewater Samples - Mallet Treatment Area</b>						
	<b>Pre-treatment</b>	<b>Day 1</b>	<b>Day 14</b>	<b>Day 28</b>	<b>Day 56</b>	<b>Total</b>
<b>Treatment Area<sup>a</sup></b>	1	5	5	5	5	21
<b>Off-plot Transect 1 (flood tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot Transect 2 (flood tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot Transect 3 (flood tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot Transect 4 (ebb tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot Transect 5 (ebb tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot locations</b>	0	2	2	2	2	8
<b>Total</b>	1	27	27	27	27	109

<b>Sediment Porewater Samples - Nuprid Treatment Area</b>						
	<b>Pre-treatment</b>	<b>Day 1</b>	<b>Day 14</b>	<b>Day 28</b>	<b>Day 56</b>	<b>Total</b>
<b>Treatment Area<sup>a</sup></b>	1	5	5	5	5	21
<b>Off-plot Transect 1 (flood tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot Transect 2 (flood tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot Transect 3 (flood tide)<sup>b,c,d</sup></b>	0	4	4	4	4	16
<b>Off-plot Drainage Stream<sup>a,b,c</sup></b>	0	4	4	4	4	16
<b>Off-plot locations</b>	0	2	2	2	2	8
<b>Total</b>	1	23	23	23	23	93

<b>Sediment Porewater Samples - Control Area</b>						
	<b>Pre-treatment</b>	<b>Day 1</b>	<b>Day 14</b>	<b>Day 28</b>	<b>Day 56</b>	<b>Total</b>
<b>Control Area<sup>e</sup></b>	1	1	1	1	1	5

**Notes:**

a - If imidacloprid concentrations are less than 0.6 µg/L in porewater samples collected from within the treatment area, samples collected at later dates will not be analyzed.

b - Samples will be collected along transects at distances of 60, 120, 240, and 480 meters from the boundary of the treatment area.

c - If imidacloprid porewater concentrations are less than 0.6 µg/L, samples collected at later dates from this same location will not be analyzed.

d - If imidacloprid porewater concentrations are less than 0.6 µg/L, samples collected more distant from this location will not be analyzed.

e - If imidacloprid porewater concentrations are non-detected, samples collected at later dates from this same location will not be analyzed.

**Table 6 - Analytical Decision Logic for Vegetation Samples**

Vegetation Samples - Mallet Treatment Area					
	Pre-treatment	Day 1	Day 14	Day 28	Total
Treatment Area <sup>a</sup>	1	2	2	2	7
Outside Treatment Area <sup>b,c</sup>	0	6	6	6	18
<b>Total</b>	<b>1</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>25</b>
Vegetation Samples - Nuprid Treatment Area					
	Pre-treatment	Day 1	Day 14	Day 28	Total
Treatment Area <sup>a</sup>	1	2	2	2	7
Outside Treatment Area <sup>b,c</sup>	0	6	6	6	18
<b>Total</b>	<b>1</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>25</b>
Vegetation Samples - Control Area					
	Pre-treatment	Day 1	Day 14	Day 28	Total
Control Area <sup>d</sup>	1	2	2	2	7

**Notes:**

a - If imidacloprid is not detected in the Day 1 eelgrass samples collected from within the treatment area, samples collected at later dates will not be analyzed.

b - If imidacloprid eelgrass concentrations are less than 10 µg/L, samples collected at later dates from this same location will not be analyzed.

c - If imidacloprid eelgrass concentrations are less than 10 µg/L, samples collected more distant from this location will not be analyzed.

d - If imidacloprid eelgrass concentrations are non-detected on or after Day 1, samples collected at later dates from this same location will not be analyzed.



**Table 7 - Analytical Decision Logic for Infauna Samples**

Infauna Samples - Mallet Treatment Area				
	Pre-treatment	Day 14	Day 28	Total
Treatment Area <sup>a</sup>	20	20	20	60
Outside Treatment Area <sup>a,b</sup>	0	32	32	64
<b>Total</b>	<b>20</b>	<b>52</b>	<b>52</b>	<b>124</b>
Infauna Samples - Nuprid Treatment Area				
	Pre-treatment	Day 14	Day 28	Total
Treatment Area <sup>a</sup>	20	20	20	60
Outside Treatment Area <sup>a,b</sup>	0	32	32	64
<b>Total</b>	<b>20</b>	<b>52</b>	<b>52</b>	<b>124</b>
Infauna Samples - Control Area				
	Pre-treatment	Day 14	Day 28	Total
Control Area <sup>a</sup>	20	20	20	60

Note:

a - If differences in infaunal abundances and species richness are not found between treatment and controls after 28 days, no further sampling and analysis are required.

b - Note, the total of 52 samples for Days 14 and 28 assumes 8 replicates will be taken at each of 4 sample locations. The actual number of sample locations will be determined on a site specific basis as discussed in the text.

**Table 8 - Sample Preparation and Analysis Methods and Quantitation Limits**

	Preparation	Analysis	Practical Quantitation
Parameter	Method	Method	Limits
Conventionals:			
Porewater Extraction	Vacuum Extraction		
Imidacloprid			
Sediment	EPA 3534C	EPA 8321B	6.7 µg/kg
Surface Water	EPA 3535A	EPA 8321B	0.02 µg/L
Interstitial porewater	EPA 3535A	EPA 8321B	0.02 µg/L
Eelgrass	AOAC Method 2007.01	AOAC 2007.01	10 µg/kg



**Table 9 - Quality Control Procedures for Imidacloprid Analysis**

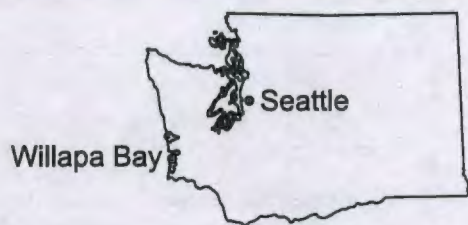
Quality Control Procedure	Frequency	Control Limit	Corrective Action
<b>Instrument Quality Assurance/Quality Control</b>			
Initial Calibration	Prior to analyzing samples with a minimum of 5 standards	Linearity (r) > 0.99	Laboratory to recalibrate and reanalyze affected samples
Continuing Calibration	Mid-range calibration standard every 10 samples	Percent difference < 15%	Laboratory to recalibrate if correlation coefficient or response factor does not meet method requirements
<b>Method Quality Assurance/Quality Control</b>			
Holding Times	Not applicable	See Table 2	Qualify data or collect fresh samples in cases of extreme holding time or temperature exceedance
Method Blanks	One per sample batch or every 20 samples, whichever is more frequent, or when there is a change in reagents	Analyte concentration < LOQ	Laboratory to eliminate or greatly reduce laboratory contamination due to glassware or reagents or analytical system; reanalyze affected samples
Analytical (Laboratory) Replicates and Matrix Spike Duplicates	One duplicate analysis with every sample batch or every 20 samples, whichever is more frequent; Use analytical replicates when samples are expected to contain target analytes. Use matrix spike duplicates when samples are not expected to contain target analytes	Compound- and matrix-specific RPD $\leq$ 35 % applied when the analyte concentration is > PQL	Laboratory to redigest and reanalyze samples if analytical problems suspected, or to qualify the data if sample homogeneity problems suspected and the project manager consulted
Matrix Spikes	One per sample batch or every 20 samples, whichever is more frequent; spiked with the same analytes at the same concentration as the LCS	Compound- and matrix-specific	Matrix interferences should be assessed and explained in case narrative accompanying the data package.
Surrogate Spikes	Triphenylphosphate added to every sample	40 – 120% recovery	Laboratory to redigest and reanalyze samples if analytical problems suspected, or to qualify the data if sample homogeneity problems suspected and the project manager consulted
Laboratory Control Samples (LCS), Certified or Standard Reference Material	One per analytical batch or every 20 samples, whichever is more frequent	13 – 133% recovery	Laboratory to correct problem to verify the analysis can be performed in a clean matrix with acceptable precision and recovery; then reanalyze affected samples

## FIGURES

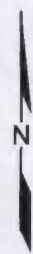


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Scale in Miles



Source: Base map prepared from DeLorme Topo 7.0, 2007.

Willapa Bay SAP  
Willapa Bay, Washington

Vicinity Map

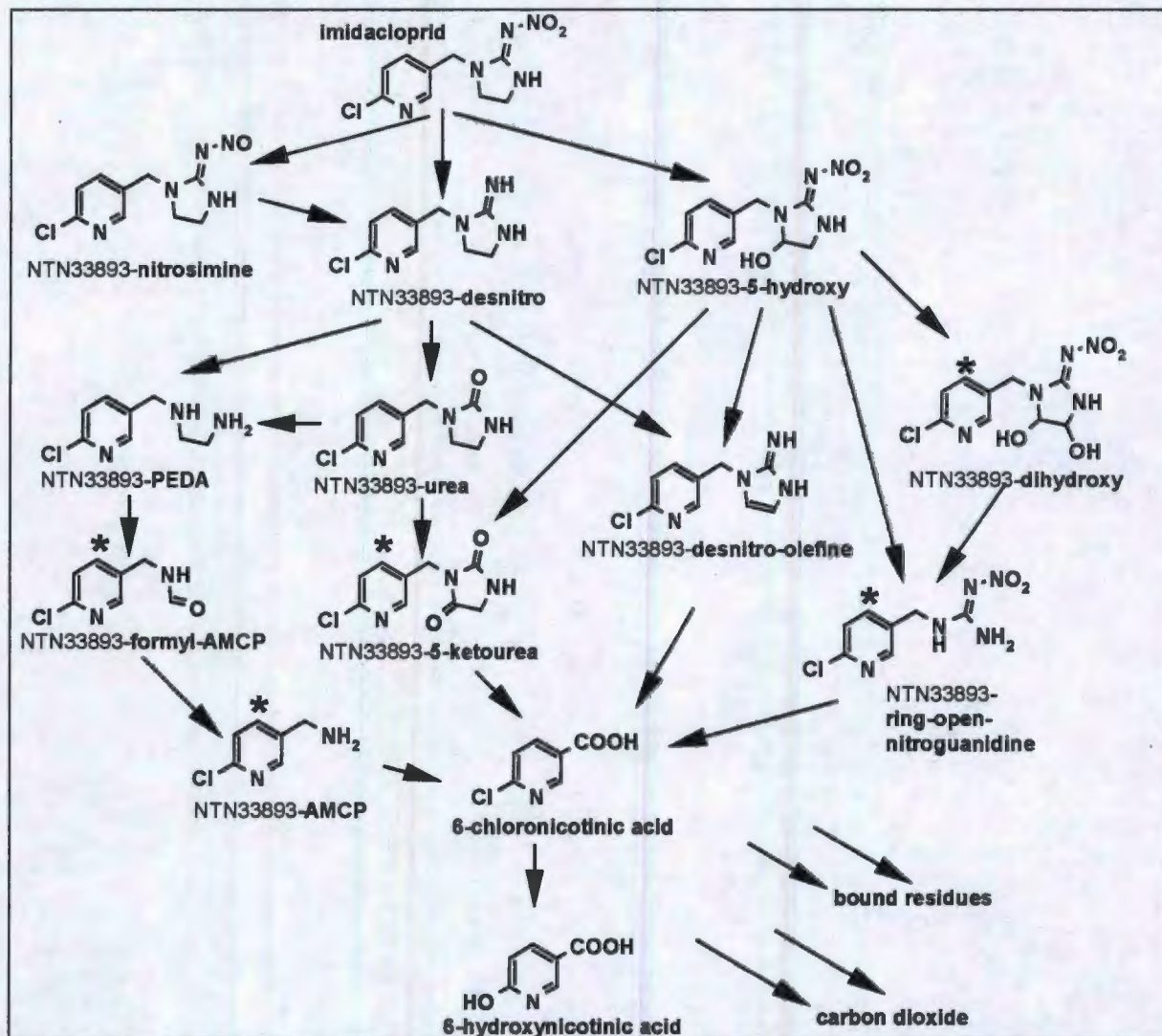
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**HARTCROWSER**

Figure

**1**



\*) Degradates marked with an asterisk were detected only in light-exposed systems.

Willapa Bay SAP  
Willapa Bay, Washington

Proposed Metabolic Pathway for Degradation  
of Imidacloprid in Aquatic Systems

12733-02

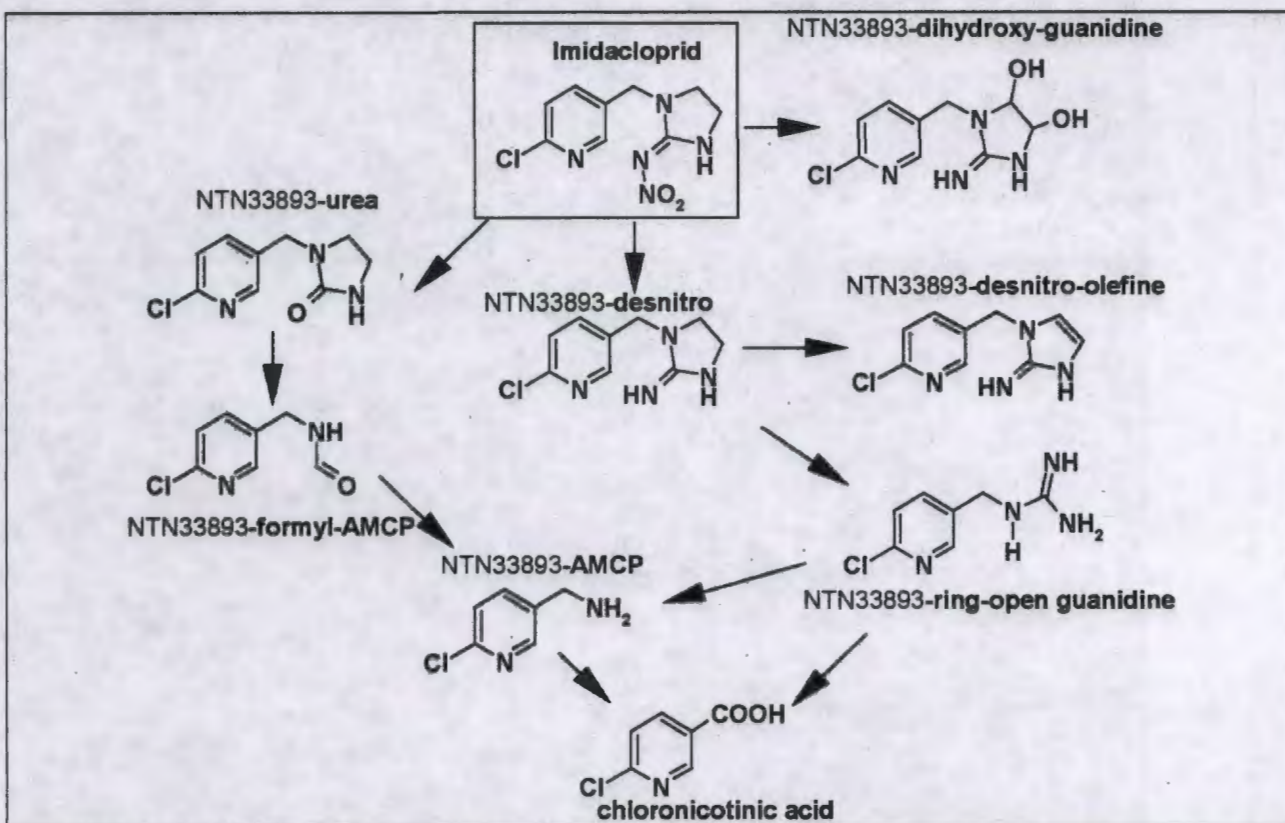
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**HARTCROWSER**

Figure

**2**





Willapa Bay SAP  
Willapa Bay, Washington

Proposed Metabolic Pathway for  
Photo-Transformation in Water  
of Imidacloprid

12733-02

3/12

**HARTCROWSER**


Figure

**3**

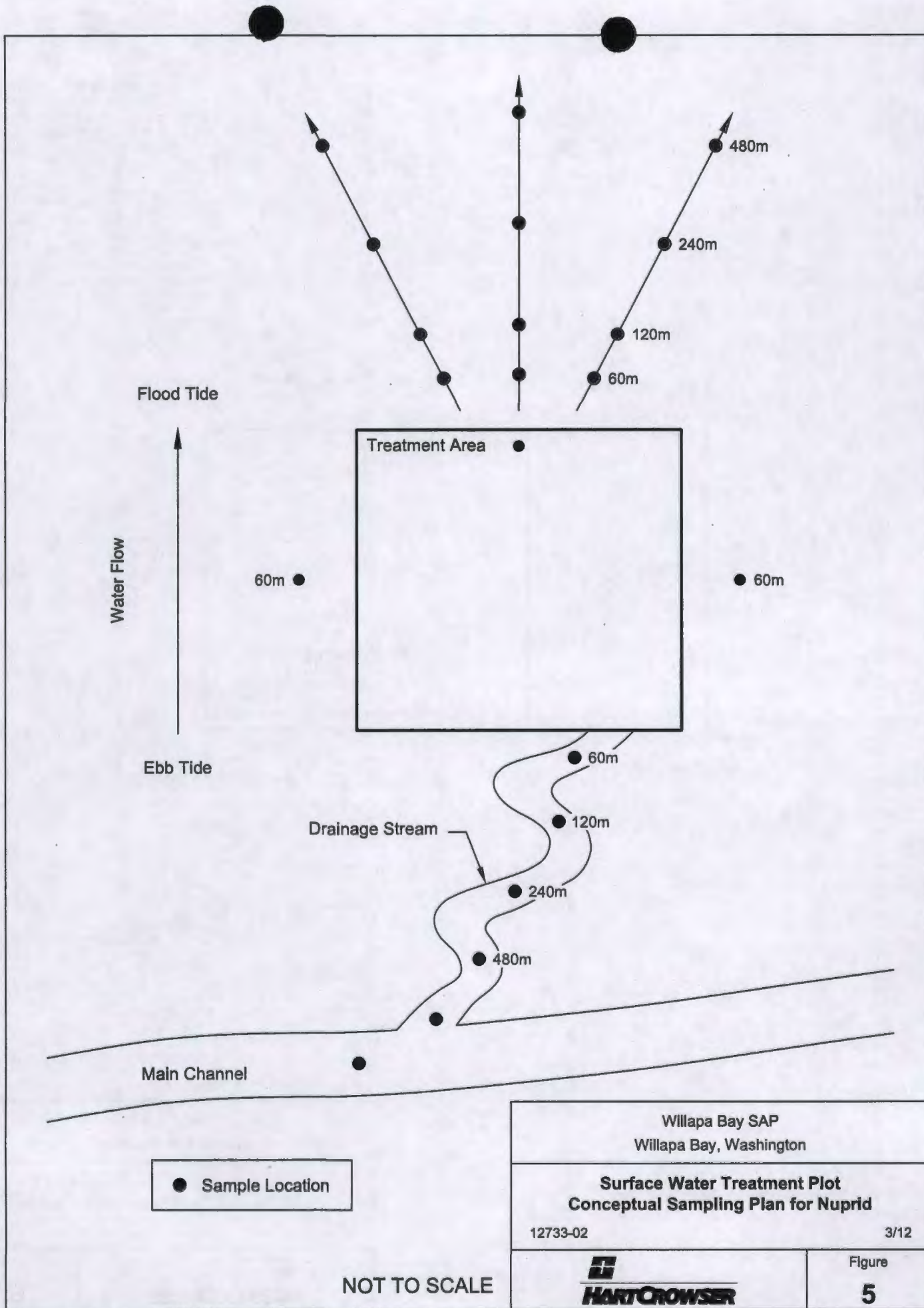




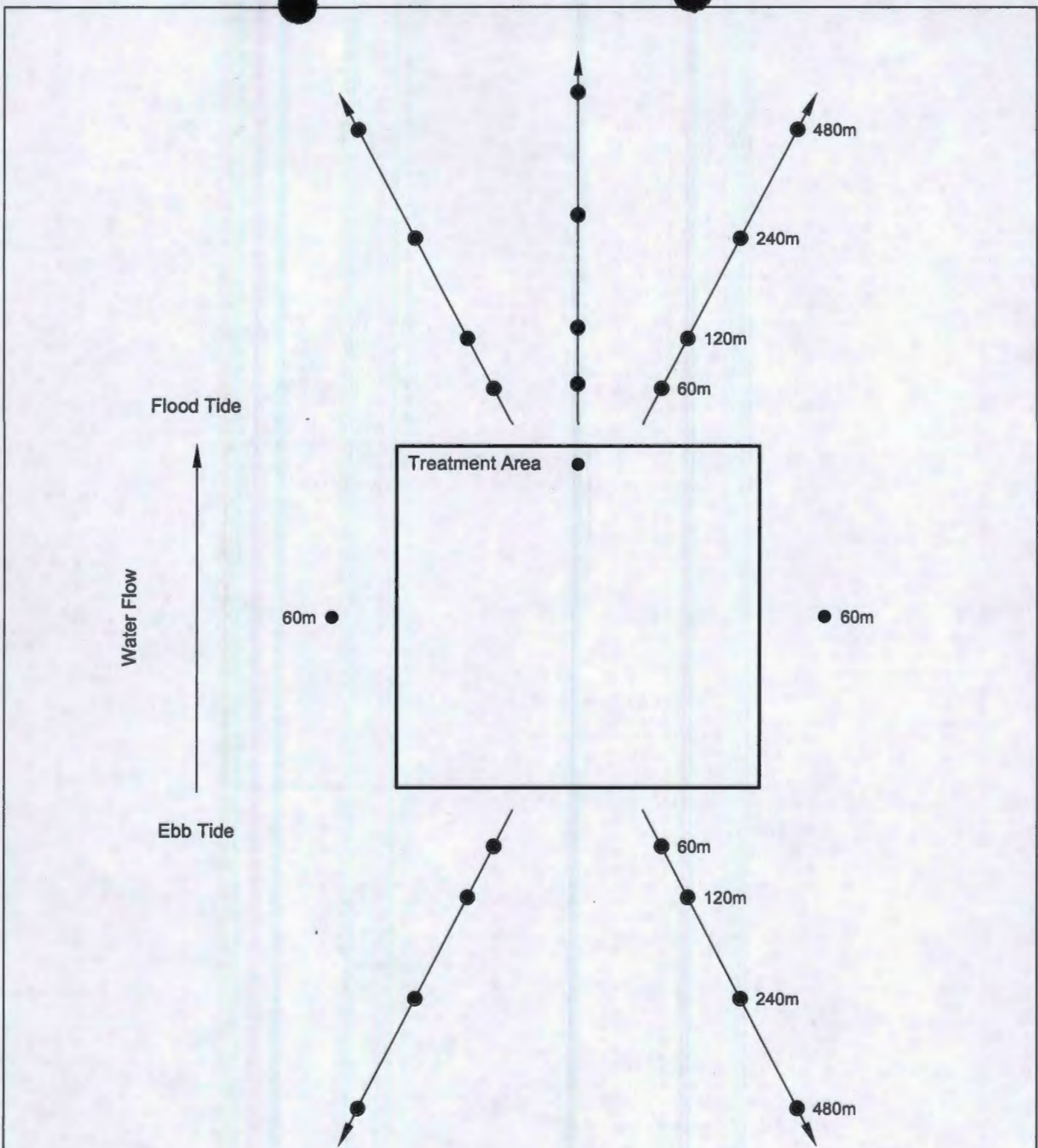
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Willapa Bay SAP Willapa Bay, Washington	
Proposed Treatment and Control Plot Locations	
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 <b>HARTCROWSER</b>	Figure <b>4</b>


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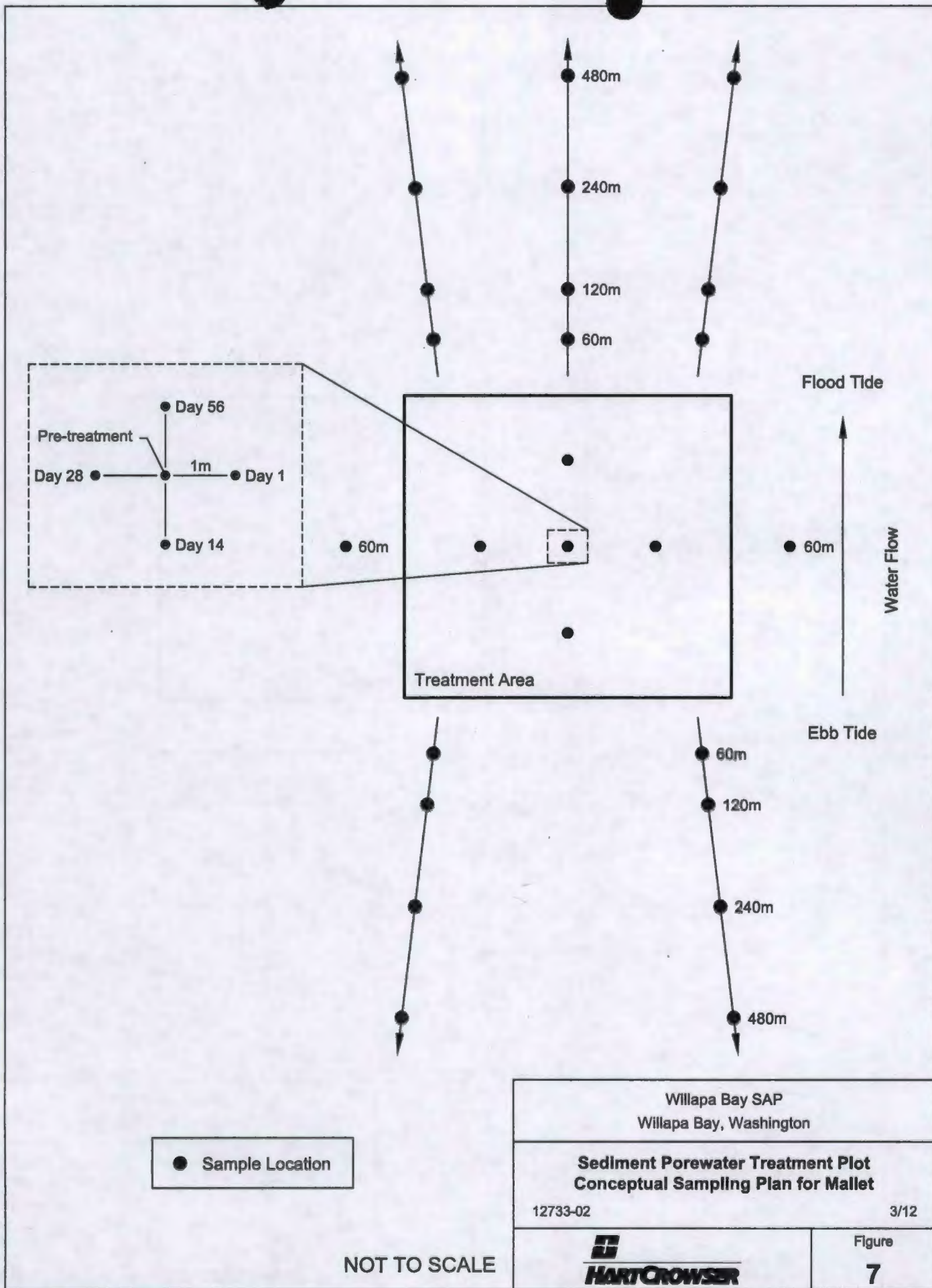
● Sample Location

Willapa Bay SAP Willapa Bay, Washington	
Surface Water Treatment Plot Conceptual Sampling Plan for Mallet	
12733-02	3/12
 <b>HARTCROWSER</b>	Figure <b>6</b>

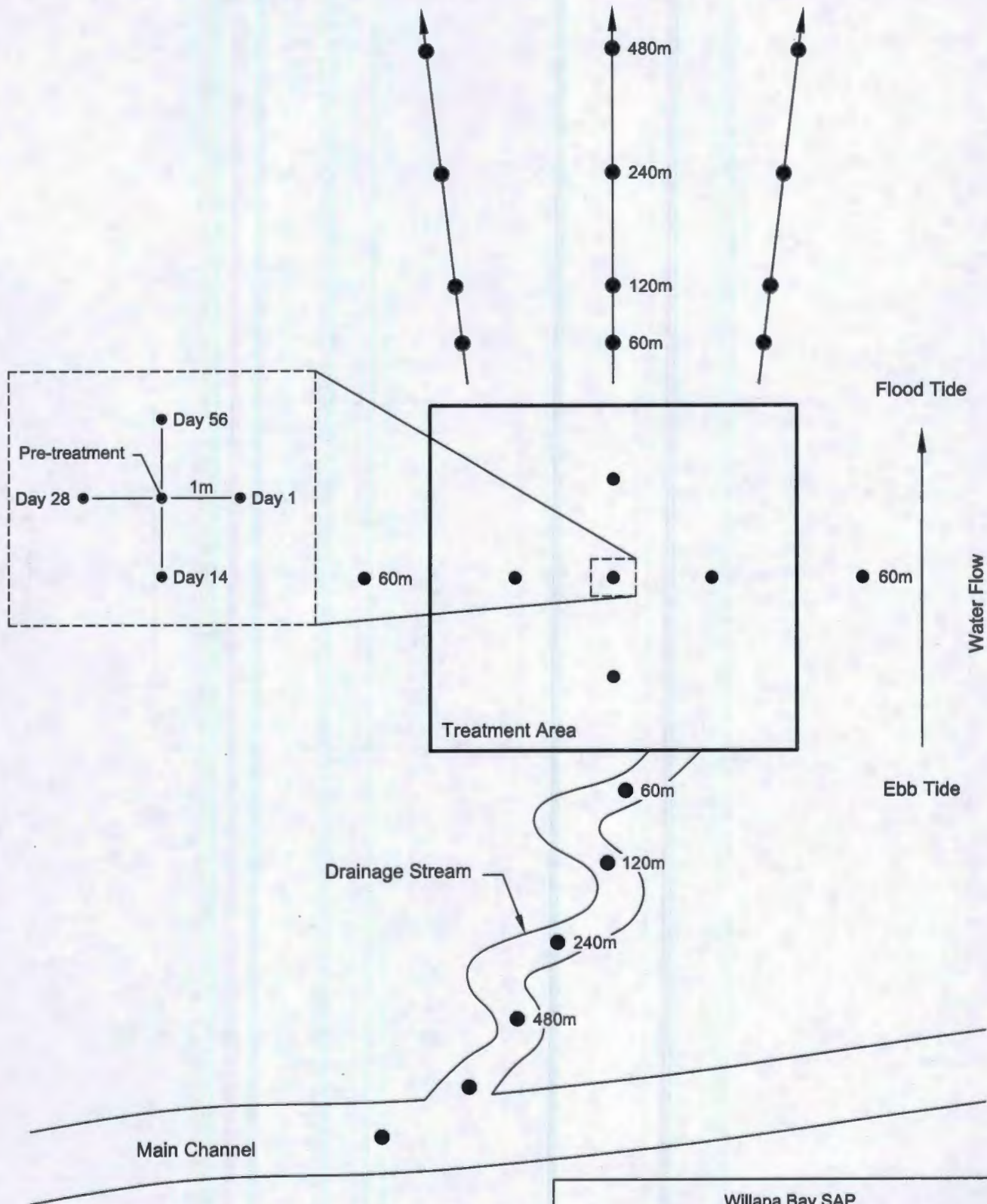
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Willapa Bay SAP  
Willapa Bay, Washington

**Sediment Porewater Treatment Plot  
Conceptual Sampling Plan for Nuprid**

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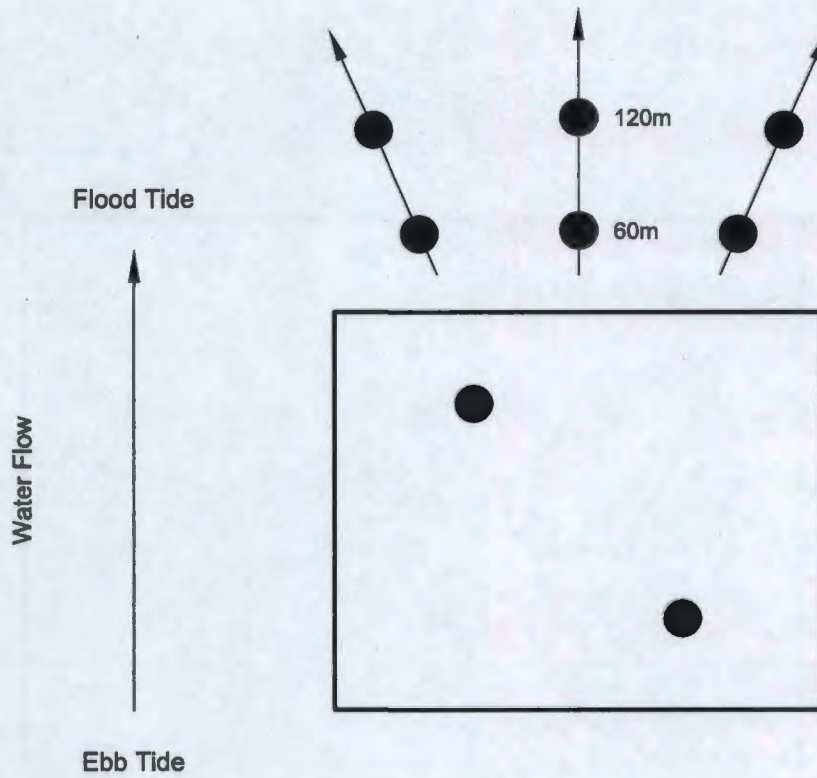
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**HARTCROWSER**

Figure

**8**





● Sample Location

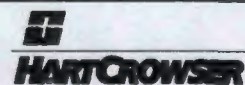
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Willapa Bay SAP  
Willapa Bay, Washington

**Vegetation Treatment and Control Site  
Conceptual Sampling Plan**

12733-02

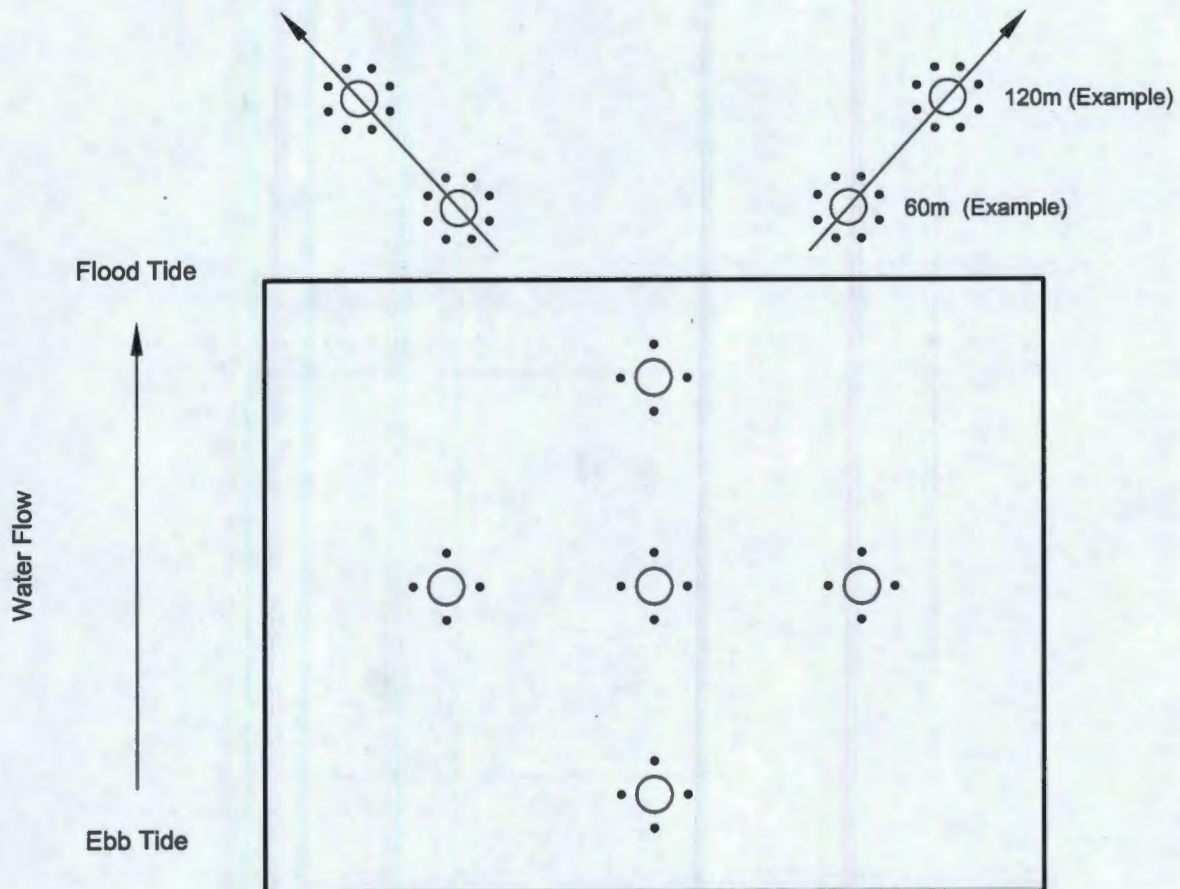
3/12



Figure

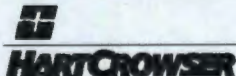
**9**





- Original Pore Water Sample Location
- Replicate Infauna Sample Location

NOT TO SCALE

Willapa Bay SAP Willapa Bay, Washington	
Benthic and Epibenthic Invertebrate Treatment Plot Conceptual Sampling Plan	
12733-02	3/12
	Figure <b>10</b>

## PHOTOGRAPHS



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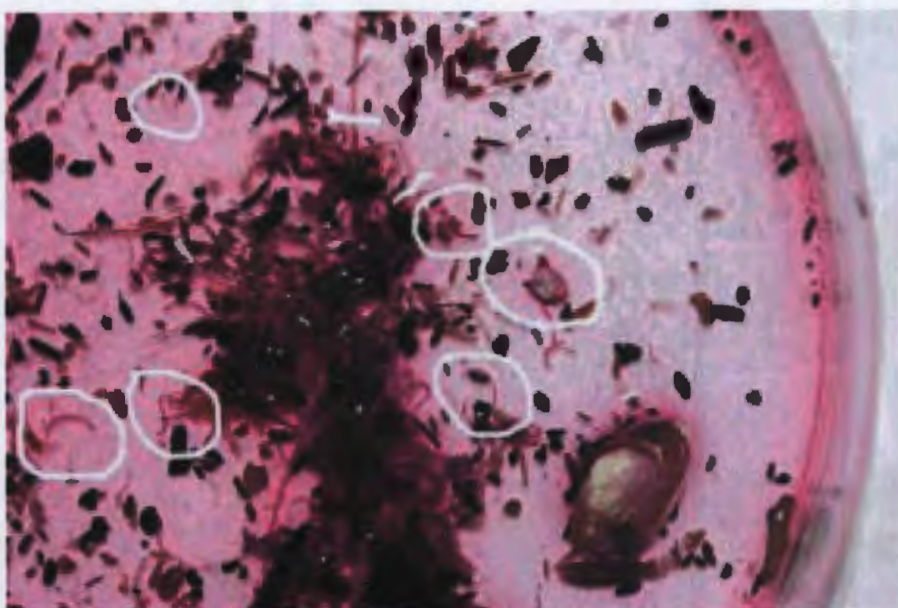
Photograph 1. Clam gun used to sample epibenthic and benthic invertebrates.



Photograph 2. Sieve bucket used to sieve epibenthic and benthic invertebrate samples.



Photograph 3. Technicians sorting organisms from sediments and detritus into vials.



Photograph 4. Small polychaetes and bivalves (circled in white) are stained with rose Bengal.



**APPENDIX B  
RESPONSES TO PREVIOUS  
EPA CORRESPONDENCE**



**Appendix B**  
**Responses to Previous EPA Correspondence**

- I. Responses to: "Review of sampling analysis protocol for the use of imidacloprid on oyster beds under an experimental use permit" DP Barcode: 391941, 391695; PC Code: 129099; Date: 08/11/11

Our response is noted in **bold type** below for each listed comment.

**Study Design**

- Page 3. The protocol states that Nuprid 2F will be applied to silty sediment and sandy partially vegetated sites. Additionally, Mallet 0.5% G will be applied to silty sediment in shallow water and sandy vegetative sites in shallow water. These two descriptions indicate possible differences between the two sites with different application methods. Therefore, differences in site characteristics may impair the comparison between endpoints of concern across methods of application and the control. For example, at Bay Center, Nuprid 2F will be applied to sandy partially vegetative bed, but Mallet 0.5G will be applied to a sandy vegetated bed. Will the control site at Bay Center be vegetated or partially vegetated? Considering only one control plot will be established at each site (Bay Center and Cedar River), the study should insure that the control site is similar to both treatment plots so that it can provide a useful comparison to the treated plots. Furthermore, the statistical design in terms of the number of plots introduces uncertainty into the study. Only one control plot will be used at each site, which means that no measure of variability will be available within the site (i.e., no measure of control plot variability within Cedar River or Bay Center).

Our study design requires that the treated tidal ground have burrowing shrimp populations greater than the economic threshold ( $10/m^2$ ) and not have any previous treatment by carbaryl or imidacloprid. In addition, these sites must be covered by a NPDES permit issued by the Washington Department of Ecology (Ecology). These experimental treatments are authorized under an existing NPDES permit held by the Willapa Grays Harbor Oyster Growers Association (WGHOGA). The permit covers treatment on beds owned by an oyster grower (as opposed to state or federal ownership). In 2010 and 2011 we struggled to find large sites (10 ac) available in the bay that meet the research criteria and have an adjacent matching untreated control site nearby. In 2011 we could not find any sites greater than 5 ac with silt sediment that had not been treated in previous years. Recent searches during daytime low tides again failed to identify large, silty sites, although a number of larger sites on sand substrates were identified. The practical constraint on finding acceptable sites is reflected in the 2012 Sampling and Analysis Plan (SAP) we submitted to EPA and Ecology.

The SAP includes maps and coordinates of the sites we propose to sample in 2012. These consist of replicate large blocks using Nuprid, Mallet, and controls on sandy sediments with variable amounts of vegetation. Please see the SAP for additional details.

- Page 4: The iterative process proposed for expanding the scope of sample collection across time and space is conceptually attractive but its success is dependent on timely



analysis and interpretation of analytical results. We would encourage the study team to submit to EPA and Washington State analytical results as they are obtained and decisions are to be made regarding expansion or reduction in the scope of the sampling.

**This is possible for some samples that have a long holding time for analysis. However, it is not feasible with water and vegetation samples that, given short holding time requirements, will require rapid decisions regarding expansion or reduction in the scope of the sampling. EPA can provide suggestions on the iterative criteria provided in Tables 4 thru 7 of the proposed SAP to help in this regard.**

- Page 5. The protocol does not differentiate when ELISA will be used as an analytical method in place of HPLC/MS. More detail should be provided on how and when each of these methods will be used. The ELISA analyses are potentially a very useful supplement to the HPLC/MS analyses but should not serve as a complete replacement analytical method. Note the following description of the ELISA method sensitivity:
  - The ELISA, although most sensitive to parent imidacloprid, also detects some of its metabolites (with less sensitivity) and is strictly speaking a nonspecific method since the proportion of the analytical response due to these derivative products is unknown. These derivatives share the imidazolidinyl moiety of the parent compound that is recognized by the binding antibodies. Differences in the structures of the imidazolidinyl ring of these metabolites from IMI result in their partial detection. The three metabolites examined, Imidacloprid Olefin, DesNitro Imidacloprid, and Imidacloprid Urea have cross-reactivities of 32, 60 and 34%, respectively. Potential cross-reactivity with other derivatives has not been reported (see Kanne et al. 2005). Measurements made with the ELISA do not differentiate between the detected concentration of IMI and these metabolites. This is in contrast to HPLC/MS that only quantifies the parent compound<sup>1</sup>.
  - Because of the cross reactivity issues with the ELISA (differentially sensitive to both compounds of interest (toxic metabolites) and compounds not of interest for this study) we strongly encourage that a core sampling set always be analyzed by an HPLC-MS or similar method for imidacloprid parent and at a minimum the olefin [1-(6-chloro-3-pyridylmethyl)-N-nitro-1,3-dihydro-imidazol-2-ylideneamine] and 5-hydroxy [1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-5-ol] metabolites. A minimum of 15% of all samples should be analyzed by both methods. The ELISA results by themselves demonstrate the likely presence or absence of imidacloprid residues of concern in a sample without providing any ability to distinguish the specific chemical mix associated with the observed response.
  - Information on recent advances in analytical methods: We are not requiring the use of another method, but do want to note that the EPA 8321B HPLC/MS method is a multiresidue method and may not be the best performing method available at the current time for the specific task of analysis of imidacloprid and its degradates (see, e.g., Lagalante and Greenbacker (2007; Flow injection analysis of imidacloprid in natural waters and agricultural matrixes by photochemical dissociation, chemical reduction, and nitric oxide



chemiluminescence detection, *Analytica Chimica Acta* 590:151-158) who review several published analytical options for imidacloprid and metabolites and total residue methods along with presenting a new flow injection analysis method for imidacloprid and its metabolites).

- Specify whether the quantification limits (0.04 ppb) of the proposed HPLC/MS method are the same for water, pore-water, and sediment sample analysis. Also specify the expected minimum detection limits.

**During the past 5 years we have made considerable efforts to quantify imidacloprid metabolites or degradation products. Despite repeated efforts, we were unable to find any commercially available source of metabolites that could be used as standards. Without standards, quantification of metabolites was not feasible.**

Recently we have located a laboratory (ChemServe) that will do a custom synthesis to produce the two metabolites that EPA requested we investigate: imidacloprid olefin [1-(6-chloro-3-pyridylmethyl)-N-nitro-1,3-dihydro-imidazol-2-ylideneamine], and 5-hydroxy imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-(nitroimino)imidazolidin-5-ol]. The state certified laboratory that will analyze our samples (Pacific Agricultural Laboratory or PAL) has indicated that if they are given the synthesized metabolites they will be able to test for those metabolites in field samples we would submit.

Because any degradation products of imidacloprid take time to develop, and given the additional costs of testing for these metabolites, our SAP limits the samples we would test. Specifically, Day 1 samples are excluded, regardless of matrix. Pore water samples taken on days 14, 28, and 56 will be analyzed on an iterative basis for these derivatives, as will vegetation (eelgrass) samples from days 14 and 28.

Based on discussions with Ecology, we also intend to develop a literature review and analysis of the imidacloprid metabolites, their toxicity and persistence relative to the parent compound, and their possible impact on biological communities in treated areas. This literature review, along with the testing for metabolites, is expected to support the agency's regulatory analysis of metabolites and their effects.

We are aware of the concerns with cross reactivity of the ELISA. Subsequent to our last submission to EPA we conducted work and prepared a report that confirms the close correspondence between the ELISA and HPLC for concentrations above about 12 ppb. We are currently examining the possibility that the observed differences between the ELISA and HPLC at later time points (and lower concentrations) in the field samples are due to a matrix effect associated with the "other" ingredients in the formulated product and not derivatives.

The work done to date on ELISA and HPLC gives us confidence that results from past work with ELISA are reasonably accurate and therefore useful for assessing the results of our field trials. For 2012, however, we have agreed with Ecology that water, sediment pore water, and eelgrass samples will all be analyzed using HPLC rather than ELISA. We have collectively agreed to this in order to eliminate any questions about



ELISA (e.g., cross reactivity) from our 2012 results.

PAL reports that the quantification limits of the proposed HPLC/MS method are 0.02 ug/l for water and pore-water, 6.7 ug/kg for sediment, and 10 ug/kg (wet weight) for eelgrass sample analyses. PAL said they do not do method detection limit studies because of the questionability of any results below the quantification limit.

- Page 5, in the “Epibenthic and Benthic Infauna” section:
  - Sampling methods need to be specified.
  - Indicate what means are referred to here: “20 core samples precisely described the means” and provide the specific reference.
  - A specific citation is needed for the analysis used to justify the replication level chosen.

Results of a power analysis (IPM SPSS Sample Power <sup>TM</sup>, Release 3.0) conducted on 2010 benthic invertebrate data showed the number of sample replicates from those studies (16 or 20 per sample date/plot) was almost always sufficient to conduct the statistical tests described above. Accordingly, we intend to use the same sampling approach in 2012. We have increased the number of replicates we would collect from each site outside of the plots to 8 per location to ensure sufficient power in the analysis of results. The SAP has also been amended to more explicitly discuss sampling methods for invertebrates.

- Page 7. The plot selection method does not allow for replication. According to the protocol, Bay Center and Cedar River sites are unique in their sediments and vegetation pattern. One plot is set up per treatment at each site. The replication is therefore at the subplot level and represents pseudoreplication, so the statistical power will be limited. Any measure of variability will be at the subplot level, and inferences to the larger bays as a whole will be extremely limited.

We concur, but large-scale replicated trials are not technically (see above on comment for Study Design) or fiscally feasible. We will use data across three years of studies on different sites to help add certainty to our results despite limited replication. We believe that by doing so, valid large bay conclusions are feasible. For some data sets (water concentration, efficacy, macrofauna impact) ample replication across years and sites is available for estimating variability.

We discussed with Ecology how best to sample in 2012. Specifically, we asked if using small plots to increase replication would be preferable to limited replication using large plots that approximate the size of future treatment areas where imidacloprid would be applied by oyster growers. After internal consideration, Ecology recommended to us that we use realistic plot sizes, even if that limited replication. Hence, for 2012, we will have two replicates each of Mallet, Nuprid, and controls, all on large plots. See the SAP for maps and additional details on these locations.

#### Sediment and Pore Water Sampling

- Pages 10 and 11. Tables 2a and 2b show that samples from 10-20 cm layer of the on-site



sediments will be taken at only 1 and 2 days after treatment. The protocol should provide a rationale why this layer of the sediment will be analyzed in the short time frame of at most 2 days after treatment. EFED recommends that sampling and analysis continue in the 10-20 cm layer for 28 days or until 3 consecutive non-detects are established, whichever is longer.

- Pages 10 and 11. Tables 2a and 2b are unclear as to what they mean by the number of samples per bed. The tables appear to not be completely labeled (e.g., why is the total number of samples two times the number of replicates x the number of sampling intervals?). This protocol description in the tables should be clarified.
- Page 11. Please cite the specific methods to be used for extraction of soil pore water and bound sediment residues.

We have revised our 2012 sampling protocol to include sediment pore water sampling at 1, 14, 28, and 56 days after treatment (DAT). We will use an iterative approach to select which sediment samples are analyzed. Spatially, we will not analyze samples from areas that water quality monitoring on the first tide following treatment does not document were exposed to screening level concentrations of imidacloprid. Temporally, we will not analyze samples from a given location on any date if sediment pore water concentrations at that location have previously tested at levels below a second screening level concentration specific to pore water. As discussed in the SAP, these water and pore water screening levels are conservative standards tied to the acute and chronic toxicity literature, respectively, for imidacloprid. Continuing to collect and analyze samples for 3 subsequent dates would require staffing and financial resources WGHOGA does not have, and verifying 3 non-detects would have limited scientific value given the overall goals of the experimental study.

We apologize for any confusion in our previously submitted tables. They have been revised in the updated 2012 SAP submitted to EPA. We intend to collect 5 sediment samples from the treated area on each date, 1 sample from the control on each date, and a variable number from outside the treatment areas dependent upon site conditions.

The SAP provides details on the methods for coring and pore water extraction. In 2011, we utilized a 0-10 and 10-20 cm sampling protocol. Initial results indicate that the 0 to 10 cm sampling stratum will provide worst case impact data, at least for sandy substrates, and that additional deep core sampling is not warranted in this substrate. Preliminary analyses of spiked muddy sediments suggest binding to sediments may be occurring, likely the organic matter. But muddy sediments are not included in the 2012 SAP for the reasons noted above.

#### Water Column Monitoring

- Page 12. The protocol identifies that the sampling for imidacloprid concentrations in surface water will be made in 20 cm of water on the first incoming tide and then high tide thereafter at the specified intervals. Sampling should also be conducted on the outgoing tide in order to assess export off site. In addition, the sampling for water column concentrations should occur in worst case conditions. Measurements at 20 cm may not be worst case conditions, but rather as the water is first moving off the bed. Finally, high tide represents the best possible scenario, whereas first tidal inundation/low tide would provide a better measure of worst-case exposure.



- Page 12. For the 5 acre treatment beds, the protocol does not state what type of sampling vessel will be used to collect on-bed water samples. EFED is assuming that 1 L amber glass bottles will also be used to collect on-bed water samples on the 5 acre treatment beds in Cedar River.
- Page 13. The protocol states that a single sample will be collected in 20 cm water depth at 240 m from the outer bed edge in the direction of the current, but only for the granular applications. A single sample provides very little ability to characterize concentrations of imidacloprid in ebb water at the edge of the plot. It is unclear as to how this information will be useful given the inability to characterize residue concentrations spatially. Furthermore, only one time point, 0.2 hours from treatment, will be used for sampling ebb water. Therefore, any time trends will also be uncertain as it relates to movement of imidacloprid off-site over time.
- Page 13. Table 3a illustrates the sampling plan for water column concentrations at Bay Center. This table shows that samples will not be collected on the water control site at 2 hours after treatment. However, table 3b, which describes the water column sampling plan for Cedar River, shows that sampling will occur on the water control site at 2 hours after treatment. Table 3a should be updated to reflect the same sampling protocol as outlined in Table 3b, or a rationale should be provided to explain the difference.

**We appreciate these comments and apologize for any confusion created by our original submittal. We have rewritten this portion of our study plan. We believe the revised SAP addresses these comments. In particular, water samples are now to be taken when the advancing tide water is 5 cm deep, rather than 20 cm. In addition, additional sampling of ebb waters is included for both the Nuprid and Mallet sites.**

#### Vegetation Sampling

- Page 14. The study protocol states that all of the vegetation samples that are collected will be taken out to deeper water and washed with bay water. A rationale should be provided as to why bay water is being used rather than collecting the vegetation samples and washing them with a pure isotonic solution. If washed off with the prepared isotonic solution, the rinsate could also be analyzed to determine the amount of residue freely removed from the plant by washing.
- Page 14. The protocol states that vegetation will be sampled from two on-bed locations where the vegetation density is high enough to justify sampling. If possible, vegetation should be sampled at the boundaries of the plot as well in order to assess the spatial edge of potential accumulation of residues. If there is any contamination of vegetation at the edges of control or treated plots, additional sampling should characterize the extent of vegetation contamination.
- Page 15. The protocol includes a procedure to sample the water column, pore water, and sediments on the control plots. However, there is no current plan as outlined by the study protocol to sample vegetation within the control plot. EFED recommends that the control plot vegetation also be sampled and analyzed to insure no contamination of control vegetation exists.

**Washing vegetation with clean bay water to remove sediment rather than an isotonic solution is preferred for several reasons.**



1. These sites are remote and accessible only after ½ to 2 miles of walking in soft mud. Carrying isotonic washing solution for rinsing, plus all the other required sampling gear, is not feasible.
2. The rinsate bay water we use is collected far enough off sites to not be contaminated.
3. Our available data on residues in eelgrass suggest levels are extremely low (below reporting limits) and not an environmental concern. Given this we do not believe that the time and expense to collect and analyze rinsate samples from the eelgrass are warranted, especially given the concurrent collection of water samples from locations at or near the sampled eelgrass.

We agree that vegetation sampling off-bed and in the control(s) is warranted, and have included such sampling in our proposed 2012 SAP.

#### Benthic and Epibenthic fauna

- Page 15. The sampling of infauna commences at 28 days post application according to Tables 4a and 4b. EFED is concerned with both acute and chronic effects. Therefore, EFED suggests that 28 days is too long of a period between application and sampling. A shorter sampling interval should be considered.
  - Page 15. Tables 4a and 4b are unclear about the number of replications per bed and the total samples. The tables should be revised in order to more clearly describe the study design.
  - Page 16. The protocol states that duplicate field samples will be collected for sediment analysis. Duplicate field samples should also be used for water column and pore water for quality control.
  - Page 16. The protocol states that epibenthic and benthic invertebrates will be sampled adjacent to the sediment sampling stations at both treated and untreated beds. However, the protocol does not clearly state which sampling stations will be sampled for these invertebrates. The protocol should clarify at which sampling stations any sampling for invertebrates will occur.
  - Page 17. The protocol states that comparisons will be made for total abundance between treated and untreated sites and among pre- and post-treatment intervals. EFED recommends all comparisons be performed between treated and a paired control site to avoid seasonal variations. These comparisons should be performed at both immediately before treatment and at various times post-treatment.
  - Page 18. The protocol states that transects will be established to assess epibenthic megafauna. How will the protocol address the potential for movement of crab and fish, among other megafauna? The protocol should provide more description as to how this data will be collected, and how this method will avoid duplicate counts of individuals. The timing of the sampling with respect to the tidal cycle and weather events may have a very substantial effect on megafauna observations; this needs to be accounted for.

Thank you for these comments. We apologize for any confusion created by our original sampling plan. We have rewritten and also expanded the scope of this portion of our SAP, and believe this revised approach addresses the concerns mentioned above regarding benthic and epibenthic invertebrates. We offer here the following specific responses:



- Our plan now includes sampling at 14 days post-treatment, as well as at 28 days.
- Method blanks and matrix spikes are now included in our analysis methods.
- We will be comparing both at sites across time (e.g., treatment sites before and after treatment), and between control and treatment sites within each time period. The former gives us the best understanding of how sites recover biologically after imidacloprid treatment. The latter provides a measure of how that recovery compares to community structure at untreated sites.
- We understand your concerns regarding duplicate counts for megafauna sampling; however, our data are collected during low tide. Fish are confined to shallow tidal pools and crab or other megafauna are not very mobile on dry tidal ground.

#### General Comments on the Protocol

The cross reactivities of the metabolites indicate that ELISA for parent imidacloprid will also detect the olefin, desnitro, and urea metabolites. However, the reactivities are 32, 60, and 34%, respectively. Therefore, the quantitative response from the ELISA will overestimate concentrations of the parent compound in samples where there is a substantial presence of the metabolites (but it cannot be known for which samples this is an issue without separate chemical-specific analysis). It is possible that in certain samples the presence of less toxic metabolites like imidacloprid urea could dominate and be responsible for the majority of the response observed with the ELISA method. According to the preliminary 2010 results from the field study report entitled, "Concentrations of Imidacloprid in Sediment Pore Water Following Application of Imidacloprid in Willapa Bay, Washington", ELISA appears to generally reflect the results from the analysis performed by HPLC/MS at 0 – 24 hours. However, at one sample point, ELISA overestimates the concentrations in pore water whereas at another sample point it underestimates concentrations. By 14 days and beyond, ELISA consistently overestimates concentrations relative to HPLC/MS, indicating that the level of the ELISA method analytical response is likely being influenced by cross-reactive degradates. These results indicate that parent imidacloprid is degrading, and that significant amounts of the metabolites remain present in pore water.

The ELISA method may provide valuable information on the presence of imidacloprid residues, but the results can only be properly interpreted when supplemented with chemical specific analyses. The olefin and the 5-hydroxy are both toxic to terrestrial invertebrates. Yet data are lacking on the toxicity of these two metabolites to aquatic invertebrates. According to the report in the Appendices on the toxicity of the metabolites as determined by in vitro methods, imidacloprid-olefin, 5-hydroxy, and nitrosimine metabolites appear to be of concern to invertebrates. Recent studies indeed demonstrate that some metabolites (olefin, 5-hydroxy) are toxic to terrestrial invertebrate species (Nauen et al. 2001; Suchail et al. 2001; Decourtye et al. 2003). Given the likely persistence of imidacloprid degradates in the pore water as reflected by the preliminary ELISA data, EFED recommends that the protocol include primary degradates of concern in the analysis.

As stated previously, this could be done through the use of the ELISA method in combination with a specified number of samples being analyzed using standard methods. For the analyses using standard methods, analyses need to be performed at a minimum for the parent imidacloprid and the olefin and 5-hydroxy degradates since they are known to be more toxic to terrestrial



invertebrates and could also be quite toxic to many aquatic invertebrate species. Both of these approaches have their respective limitations: with the ELISA approach the relative amount of cross-reactive degradates cannot be determined. Furthermore, not all of the degradates of interest can be quantified. The limitation in the chemical specific analyses (for example of olefin and 5-hydroxy) is that the amounts of other potentially toxic degradates not on the analyte schedule cannot be determined. Nonetheless, the ELISA approach can provide useful information, given analyses with standard methods to confirm residue levels as identified with ELISA, for the samples in which degradation is likely to be minimal and the parent compound accounts for majority of the analytical response. Given the above discussion, the ELISA would be most useful during the early phase of the study when degradation contributes the least to dissipation, whereas the importance of the standard methods increase in importance as time since application increases (with ELISA analytical results viewed more as an indicator of presence or absence of imidacloprid residues of concern).

**We understand the potential scientific value from analysis of metabolites. We refer you to our previous discussion on this topic, and re-emphasize that we believe we have now found a source for laboratory standards that will allow us to test for the two target metabolites identified by EPA in sediment and eelgrass samples collected after day 1.**

**We are currently examining the possibility that the observed differences between the ELISA and HPLC at later time points (and lower concentrations) in the field samples are due to a matrix effect associated with the "other" ingredients in the formulated product and not derivatives. These results will be provided and used to help interpret results at later time points and to help assess the utility of ELISA. As noted above, we have agreed with Ecology to analyze 2012 field data using HPLC methods, but feel previous year's work with ELISA constitutes a useful body of information for assessment of imidacloprid levels.**

The protocol outlines various methods for sediment collection that reflect the use of non-standard methods. The protocol should utilize standard methods for collection of samples and the analysis of those samples.

**In our discussions with Ecology we reviewed our sampling methods in some detail. In some cases we agreed that our existing methods can be continued. For example, based on data that we provided, there was agreement that sediment samples could be collected using plastic (Nalgene) containers, without impacting imidacloprid residues. But in other cases we agreed to change our methods. Use of HPLC instead of ELISA is one such change. Another is agreement that we will not freeze any sediment samples prior to pore water extraction. If holding limits are at risk of being exceeded, we will extract pore water from remaining sediments and then freeze these pore water samples or hold them at 4 degrees Celsius until they are analyzed.**

**We refer you to the SAP for a complete list of the methods we intend to use in 2012.**

EFED recommends that the focus of the sampling should be on concentrations moving off-site after the first tidal inundation and sampling should continue until 2 consecutive non-detects. While the focus should be on the first tidal inundation in order to assess the maximum exposure, continued sampling is necessary to determine the extent of potential chronic exposures in the



water column, pore water, and sediment. Hence the sampling should continue until consecutive non-detects.

**We have developed an iterative protocol in response to this request. We have expanded the range of our water sample collection to detect the extent of off-site movement in the first flood tide. And we intend to use floating materials to guide our water collection to areas that are receiving the most flow off of the imidacloprid treated areas. We believe this improves the overall sampling design, and that our data should provide a worst case analysis of imidacloprid exposures for the plot sizes selected.**

**We do not agree that sampling on subsequent tides would justify the staff time or expense. Due to dilution, imidacloprid collected in water samples in any subsequent flood tides will be many orders of magnitude lower than that found with the first flood tide. Willapa Bay has one of the largest tidal prisms of any U.S. estuary (Banas, N. S., and B. M. Hickey. 2005. Mapping exchange and residence time in a model of Willapa Bay, Washington, abranched, macrotidal estuary. J. Geophys. Res., 110, C11011, doi:10.1029/2005JC002950). In addition, a tidal range of 10 feet or more is likely from the extreme low tides when sampling will be completed to the subsequent high tide.**

Page 12. The sampling design should include transit stability samples that follow the protocol for the samples through storage and analysis to determine the impacts that handling and storage have on the stability of imidacloprid residues. As currently outlined, the transit stability for sediments will be assessed to determine the effect of the storage vessel and freezing. Water column samples should also be assessed for transit stability.

**We understand the concerns over sample stability, and have investigated this concern. In particular, we have researched concerns associated with freezing and the use of plastic containers. This work did not document degradation in the field and during transit. In part, this likely reflects our quick processing of samples in the field, and delivery to the laboratories. Water samples are collected in amber glass bottles and immediately placed on ice in a cooler. Sediment and eelgrass samples are immediately placed on ice in a black plastic bag in a cooler. Samples are transported to the laboratories either on ice in sealed coolers or frozen in coolers.**

**Between the results of our examination of this issue, and our intention to continue quick sample processing and delivery to the laboratories, we believe the existing protocols eliminate concerns associated with photo- or biological degradation prior to chemical analyses.**

**II. Responses to EPA memorandum "Experimental Use Permit for Imidacloprid Products Protector 2F and Protector 0.5G for Control of Burrowing Shrimp on Oyster Beds in Washington State." DP Barcode: D384152; PC Code: 129099;**

Our response is noted in bold type below for each listed comment.

- **Page 4. Data should be submitted to clearly characterize the level of imidacloprid in**

sediment and overlying water up to 28 days post-treatment. Data from the study should provide a clear characterization of any marginal change in the expression of imidacloprid in overlying water and sediment resulting from both granular and spray applications. The sediments should be clearly characterized in order to assess differences related to levels of imidacloprid residues.

**We have modified the proposed research plan for 2012 to include testing for imidacloprid in sediment pore water at 1, 14, 28, and 56 days after treatment (DAT) in plots exposed to both liquid and granular formulations. This portion of our plan appears to address EPA's recommendation. However, we do not propose to sample water other than on the day of treatment. As outlined in our response above, the large tidal exchange volumes in Willapa Bay will result in dilutions of many orders of magnitude with the first tidal cycle. Given limited staff and financial resources, our sampling plan generally tries to avoid collecting samples that will provide limited information on imidacloprid exposure pathways.**

- Page 4: Data to characterize the background levels should include information on prior applications (rates and timing) believed to have led to the measured background levels.

**We try to pick sites that have had no prior exposure to imidacloprid, and so do not expect background levels to be present. However, we have modified the SAP to include collection of water and sediment samples from each treatment area prior to exposure with imidacloprid.**

- Page 4: Submitted protocol must define all application parameters used for the study and should include but not be limited to:
  - Weather conditions during applications should be noted.
  - Tide conditions during applications should be noted.

**We appreciate this comment and agree all applicable conditions present at the time of application should be noted. We have regularly recorded weather and tidal conditions during field trials and will continue to do so. Time of day, field personnel, equipment used, and other parameters are also recorded. Please see the SAP for additional details.**

- Page 4: Residue collection regime must be defined in the submitted protocol and should include, but is not limited to the following:
  - Residues to be collected must be defined in the protocol and must include parent and any primary degradates.
  - Residues samples of the different matrices must be concurrent.
  - Collection regime must define exposure through 28 days posttreatment and must indicate whether imidacloprid residues are increasing, decreasing, or remaining constant during the period of assessment.

**We believe the revised SAP addresses these concerns. Specific items of note are provided below.**

**Sediment for pore water samples will be collected at 1, 14, 28, and 56 DAT. As a result, data on imidacloprid concentrations, and the time-related changes in those concentrations, if any, will be determined under our proposed research plan.**



Sediment samples will be collected concurrent with invertebrate samples at 14 and 28 DAT, allowing correlations between these two datasets. As noted, water samples other than on the day of treatment are not proposed for collection because of physical dilution from high tidal volumes in Willapa Bay. For invertebrates, we are unable to differentiate between invertebrates killed by imidacloprid from those killed by the formalin used to preserve samples in the field until enough time has elapsed for visible decomposition of imidacloprid killed animals to occur. Our experience is that this is approximately 14 days. This, in combination with the synchrony of low tides at 14 day intervals, explains the 14 and 28 DAT sample timing for invertebrates, and the concurrent sediment samples.

As noted above, we will be testing for the two imidacloprid degradation products identified by EPA in sediment pore water samples and eelgrass samples collected after day 1.

- Page 4: Residue samples for the parent imidacloprid analysis should be taken from the treatment site (from the sediment and dissolved and suspended sediments from the overlying water). Samples should be taken prior to treatment and at least twice in the first 48 hours at times during the tide cycle when sampling is feasible. After the first 48 hours sampling should continue at 4, 7, 14, 21, and 28 days post-treatment, or until 3 consecutive sampling intervals result in a non-detect.
  - The minimum detection limits for imidacloprid in water should be 5 ng/l or lower.
  - The minimum detection limits for imidacloprid in sediment should be 5 ng/l or lower.

Our modified research plan includes sampling of sediments and water within 1 DAT. Sediments are subsequently collected at 14, 28, and 56 days. We understand that sampling at more frequent intervals would provide more data points to define the trajectory of imidacloprid concentrations following treatment. However, our past work has not demonstrated such a rapid decrease in concentrations that multiple samples over the first few days after treatment are critical to defining the concentration trajectory.

In addition, low tides necessary for sampling do not occur daily. Instead each lunar month has two periods (around the new moon and full moon) when very low tides occur, and two periods (around 7 days following the new and full moon) when low tides are not very low. We therefore have to set sampling dates at those times when water levels will be low enough to provide access to the treatment and control sites, regardless of whether this interval is optimal for defining the trajectory of concentrations over time. This is one of the challenges of testing imidacloprid in an estuarine environment. Similarly, unlike farms, laboratories, or a number of other field sites, access to our sites is not facilitated by roads, or even stable, solid substrates. Instead our field crews have to walk across mud and water for distances of a half mile or more carrying all necessary sample collection materials and equipment. On arriving at the site they have to "race the tide" to get the samples before the tide returns.

We contacted our analytical laboratory (Pacific Agricultural Laboratory), an Ecology certified analytical laboratory, to determine their level of quantification (method reporting limit). They state that it is 0.020 ug/l for water and pore water, 6.7 ug/kg for sediment, and 10 ug/kg (wet weight) for eelgrass. PAL said they do not do method detection limit studies



because of the questionability of any results below the quantification limit. In any case, our laboratory does not report that they can achieve a "minimum detection limit of 5 ng/l." We will use the lowest level the laboratory is able to achieve for a certified result.

- Page 5: Sampling techniques should follow those successfully used in previously published peer-reviewed literature or recommended in EPA or OECD study guidelines. (*Document list omitted for simplicity*)

We appreciate the list of documents provided by EPA, and have incorporated elements of some of them into our proposed SAP. For the SAP we focus on the major elements of experimental design and sample collection to ensure EPA concurrence with these critical elements of our proposed work.

- Page 5: Study design must include spiked standard and negative controls and analysis requirements.

The SAP has been modified to include spiked standards and blanks.

- Page 5: Sediment samples for non-target invertebrate populations (including assessment of species composition, abundance, and diversity) should be taken pretreatment and at a minimum of 1 and 2 weeks posttreatment, in additions to the 4 weeks, 3 months, and 9 months as indicated in previous EPA communications.

As noted above, the 14 DAT timing for invertebrate samples reflects our experience that we cannot differentiate between invertebrates killed by imidacloprid and formalin used to preserve the samples in the field until approximately 14 DAT. Sampling at 7 days could also have the problem of insufficiently low tides to permit sample collection, as also noted above.

We agree with another set of invertebrate samples at 28 DAT. However, our experience based on invertebrate sampling in 2010 and 2011 is that due to seasonal changes in invertebrate populations, and particularly the influx of a great number of new organisms, differences between controls and treatment areas are minimal or absent by 28 DAT. This is good news, in that it documents rapid recovery of invertebrate populations after treatment with imidacloprid. But it also means that samples at 120, and 270 days will not yield substantial new information, but they will cost considerable staff time to collect, and time and expense to have picked and identified. Consequently our SAP does not propose to collect invertebrate samples beyond 28 DAT. We do expect that preliminary results of at least some of the 28 DAT samples would be available in time to schedule sampling at later dates if notable differences between the invertebrate populations on treatment and control sites were evident at 28 DAT.

- Page 5: Replicate sampling from replicates plots is encouraged. We would also encourage as much separation as is practicable between treatment plots and between treatment and control plots.

**These comments are noted and appreciated. As we discussed above, we plan to have 2 replicates each of Mallet, Nuprid, and controls on large plots that approximate the size of areas that would be treated by oyster growers if imidacloprid is ultimately approved for burrowing shrimp control.**

**We concur with the desire to separate treatment and control plots as much as practicable.**



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460



OFFICE OF  
CHEMICAL SAFETY AND  
POLLUTION PREVENTION

Kim Patten  
Washington State University  
Long Beach Research and Unit  
2907 Pioneer Rd.  
Long Beach, WA 98631

APR 24 2012

Dear Mr. Patten:

Subject: Request for extension of experimental use permit to use imidacloprid against burrowing shrimp  
Mallet 0.5G, EPA Reg. No. 228-484  
EPA Experimental Use Permit No. 86414-EUP-1  
New Effective Dates: April 24, 2012 to April 23, 2013  
Quantity Authorized: 15 pounds of active ingredient per year applied to a maximum of 30 acres

On the basis of the information furnished by the applicant and the annexed program, an Experimental Use Permit (EUP) under Section 5 of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (86 Stat. 983), is hereby extended for the named pesticide. Shipment/use under this Permit is subject to the provisions of 40 CFR 172.

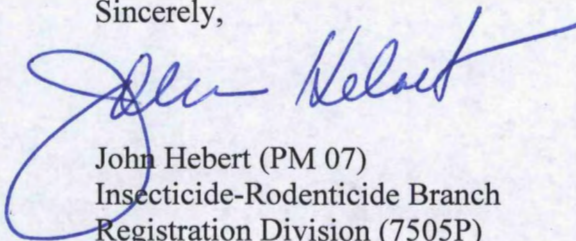
Prior to continuance of this experimental program beyond the original expiration date in any State, you are to notify the State lead agency of the States in which your experimental program will continue to be conducted of the specific testing program (when, where, how much, etc.).

Prior to the shipment/use of this material, you must consult with the state pesticide regulatory official of the states in which your experimental program will be conducted and obtain a state permit or license if such is required. Issuance of this federal permit does not negate the need for permission from individual states. Failure to do so may result in revocation or modification of this experimental use permit.

Based upon the experimental program submitted, this product may be shipped for use under this permit to Washington for use in Willapa Bay and Grays Harbor.

The labeling submitted in connection with the application for an EUP is acceptable. This labeling must be used for all shipments under this experimental use permit.

Sincerely,

A handwritten signature in blue ink, appearing to read "John Hebert", is written over a light blue rectangular background.

John Hebert (PM 07)  
Insecticide-Rodenticide Branch  
Registration Division (7505P)

Enclosure



**NUPRID 2F FOR EXPERIMENTAL USE ONLY**

Experimental Use Permit Number: 86414-EUP-1

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN THE EXPERIMENTAL USE  
PROGRAM**

---

Permittee: Kim Patten, Extension Specialist,  
Professor Washington State University Long  
Beach Research and Unit 2907 Pioneer Road  
Long Beach WA 98631

---

**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine . . . . .21.4%

OTHER INGREDIENTS: . . . . .78.6%

TOTAL: . . . . .100.0%

Contains 2 pounds of imidacloprid per gallon.

**KEEP OUT OF REACH OF CHILDREN CAUTION – CAUCION**

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle. (If you do not understand the label, find someone to explain it to you in detail.)

**ACCEPTED**

EPA Permit No. 86414-EUP-1

For shipment and use of product for experimental  
purposes under the provision of the Federal Insecticide,  
Fungicide, and Rodenticide Act, subject to attached  
comments.

Permit No. 86414-EUP-1

Issued on 4/24/12

<b>FIRST AID</b>	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>NOTE TO PHYSICIAN</b> No specific antidote is available. Treat the patient symptomatically.	

### PRECAUTIONARY STATEMENTS HAZARDS TO HUMANS AND DOMESTIC ANIMALS CAUTION

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

### PERSONAL PROTECTIVE EQUIPMENT (PPE) Applicators and other handlers must wear:

- Long-sleeved shirt and long pants
- Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
- Shoes plus socks
- Protective eyewear when working in a non-ventilated space Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS** When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

### PERSONAL PROTECTIVE EQUIPMENT (PPE)

#### Users must:

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
  - Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

### DIRECTIONS FOR USE

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:** To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Nuprid. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application. **Do NOT formulate this product into other end-use products.**

### APPLICATION INSTRUCTIONS

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 0.5 lb a.i./ac using the following properly calibrated application equipment:

- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi- amphibious vehicle (Argo™) at ~ 20 gpa.

### RESTRICTIONS:

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500 ft.
- For aerial and ground-based topical



applications and ground-based subsurface injection, all applications must be on beds exposed at low tide.

- All applications must occur between May 1 and October 15.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter ( $\frac{1}{4}$ ) mile and all public boat launches within a one-and-a-half ( $1\frac{1}{2}$ ) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth ( $\frac{1}{4}$ ) of an inch high. Signs will include a map of the inlet that wide. Signs shall be a minimum of  $8\frac{1}{2} \times 11$  inches in size, and be made of a durable weather-resistant, indicates the location of the treated area and an extended buffer that extends one-fourth ( $\frac{1}{4}$ ) mile the area's perimeter and the statement "Do Not Fish, Crab, or Clam within  $\frac{1}{4}$  mile of area treated with experimental material, as indicated by the circle on the map". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

### **SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.

### **Restrictions During Temperature Inversions**

Because the potential for spray drift is high during temperature inversions, do not make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing,

which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

### **Importance of Droplet Size**

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

### **Mixing and Loading Requirements**

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.


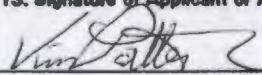
### **STORAGE AND DISPOSAL**

Do not contaminate water, food, or feed by storage or disposal. Pesticide Storage: Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. For containers smaller than 5 gallons: Non-refillable container: Do not reuse or refill this container. Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container  $\frac{1}{4}$  full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. Then offer for recycling or

reconditioning, or puncture and dispose of in a sanitary landfill, or by other procedures approved by State and local authorities. Plastic containers are also disposable by incineration, or, if allowed by State and local authorities, by burning. If burned, stay out of smoke.



Form Approved. OMB No. 2070-0040.

 <div style="display: inline-block; vertical-align: middle;"> <b>United States</b>  <b>ENVIRONMENTAL PROTECTION AGENCY</b>          Washington, DC 20460       </div>		OPP Identifier Number
Office of Pesticides Programs (7505C) <b>Application for Experimental Use Permit to Ship and</b> <b>Use a Pesticide for Experimental Purposes Only</b>		
<b>1. Type of Application</b> <input type="checkbox"/> New <input type="checkbox"/> Amendment (See No. 2) <input checked="" type="checkbox"/> Extension (Give Permit Number below)	<b>2. Briefly explain (attach a separate sheet if necessary)</b> This EUP is to be used to investigate the efficacy and non-target impacts of imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor, Washington	
Permit Number 86414-2		
<b>3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be issued (include Zip Code) (Type or Print)</b> Kim Patten, Extension Professor Washington State University Long Beach Research and Extension Unit 2907 Pioneer Road Long Beach WA 98631	<b>4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (include Zip Code) (Type or Print)</b> Nufarm Americas Inc 150 Harvester Dr, Suite 200 Burr Ridge, IL 60527	
EPA Company Number 81959-22	<b>6. Is Product Registered with EPA?</b> <input type="checkbox"/> No <input checked="" type="checkbox"/> Yes (Give Registration Number or File Symbol below) Registration Number 228-501 File Symbol	
<b>5. Name of Product</b> Mallet 0.5 G		
<b>7. Total Quantity of Product Proposed for Shipment/Use</b> Pounds of formulated product 3,000 Pounds of active ingredient 15	<b>8. Acreage or Area to be Treated</b> 30	<b>9. Proposed Period of Shipment/Use</b> 2/2012 to 6/2012 (Ship) 5/1/2012 to 10/15/2012 (Use)
<b>10. Places from which Shipped</b> Nufarm Inland Empire District 1211 St Helens St, Pasco WA 99301	<b>11. Crop/Site to be Treated</b> Oyster and Manila Clams, Willapa Bay and Grays Harbor WA	
<b>12. Specify the name and number of the contact person most familiar with this application.</b> Kim Patten 360-642-2031	<b>13. Signature of Applicant or Authorized Firm Representative</b> 	
	<b>14. Title</b> Professor, WSU	<b>15. Date Signed</b> 12/01/2011
<b>Certification</b>		
This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.		
I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.		
Below for EPA Use Only		
In any correspondence on this application, refer to this number:		Received by: EPA OPP Registration Division Washington, DC 20460
Normal review time indicates that processing of this application should be completed by (date)		
Name of EPA Contact Person	Telephone Number	

EPA Form 8570-17 (Rev. 1-94) Previous Editions are Obsolete

December 08, 2011

Jennifer Urbanski, Ph.D., Biologist  
Insecticide-Rodenticide Branch, S7221  
Registration Division (7505P)  
U.S. Environmental Protection Agency  
1200 Pennsylvania Ave. NW  
Washington, DC 20460

Dear Dr. Urbanski:

Please find enclosed our request to renew our Federal EUP for the use of imidacloprid in Willapa Bay WA in 2012. The package contains: 1) An appendix with project justification and background, 2) Forms 8570 for Mallet & Nuprid, 3) Labels for Mallet & Nuprid, 4) Final results from the 2010 season and preliminary results from the 2011 season, and 5) a new research plan for 2012.

We are in receipt of EPA's 08/11/11 comments on our Sampling and Analysis Plan entitled "Review of sampling analysis protocol for the use of imidacloprid on oyster beds under an experimental use permit, DP Barcode: 391941, 391695; PC: 129099." The attached draft of our research plan for 2012 addresses many of EPA's comments. Our final Sampling and Analysis Plan (SAP), which will be responsive to EPA's comments, will be submitted to the Washington State Department of Ecology for approval and EPA for review in the first quarter of 2012. Research conducted in 2012 will be pursuant to the approved SAP.

Do not hesitate to contact me if you have questions regarding the contents of this request.

Sincerely,



Kim Patten, Extension Professor  
WSU Long Beach Research and Extension Unit  
2907 Pioneer Road, Long Beach WA 98631  
W 360-642-2031 C 360-355-7864

2907 Pioneer Road, Long Beach, WA 98631  
360-642-2031 • Fax: 360-642-2031 • pattenk@coopext.cahe.wsu.edu

Cooperating agencies: Washington State University, U.S. Department of Agriculture, and Washington counties. Cooperative Extension programs and employment are available to all without discrimination. Evidence of noncompliance may be reported through your local Cooperative Extension office.



## ATTACHMENT 1

### Explanation and Justification

Two indigenous species of burrowing shrimp severely impact both the mudflat community and oyster production in Willapa Bay and Grays Harbor, WA. Both ghost shrimp (*Neotrypaea californiensis*) and mud shrimp (*Upogebia pugettensis*) reside in burrows beneath the mudflat surface, where they abrogate habitat from other benthic organisms and severely disrupt the structure of the mudflat substrate by bioturbation, causing cultured and native bivalves to sink and die. Although indigenous, both species, but particularly ghost shrimp, have greatly increased in density and distribution in the last 60 years, likely due to a combination of factors including loss of seasonal freshwater influx since the damming of the Columbia River and a decrease in key predators due to over-fishing.

Since the 1960s, applications of carbaryl (Sevin® 80SP, Bayer Corp.) on selected and legally limited acreage of commercial oyster beds, have effectively suppressed burrowing shrimp. A single application usually sufficed through multiple years of oyster development. A suite of best management practices, such as seasonal placement of carbaryl to avoid migratory salmon and pre-season monitoring of target beds, ensured that the estuarine ecosystem was not significantly affected. However, the potential impact of many conventional (i.e., organophosphate and carbamate) pesticides has been questioned by a variety of groups, calling into question the ability of shellfish growers to use carbaryl to control burrowing shrimp in Willapa Bay and Grays Harbor beyond 2012.

Without the ability to manage burrowing shrimp, a significant portion of the local shellfish industry would no longer be economically viable. In 1990, oyster aquaculture accounted for one of every twelve jobs in Pacific County. Since then, the decline in marine fisheries has made the local economy even more dependent on shellfish production. As demonstrated elsewhere, the collapse of agricultural and other resource-based industries often leads to increased private development and pollution.

Efforts by the Willapa Bay / Grays Harbor Oyster Growers Association (WGHOGA) to develop an IPM program have been ongoing since the inception of the carbaryl-based program, but were formalized in 2001 when a memorandum of agreement was signed with several organizations and state agencies to develop an IPM program. Investigations of alternatives to carbaryl currently involves dozens of scientists, extension agents, and grower-collaborators who focus on biological, mechanical, and chemical controls, as well as a better understanding of burrowing shrimp ecology. At this point, we have identified only a single alternative tactic, imidacloprid, that has sufficient efficacy, environmental compatibility, and potential for registration to control burrowing shrimp and allow shellfish farming to continue in Southwest Washington beyond 2012.

Small and large plot trials of imidacloprid have been conducted between 2008 and 2011. Results of those trials (2008 to 2010) have been submitted in our previous federal EUP applications and results of more recent trials (2010 and 2011) are attached as part of this submission. These studies have been useful to obtain data on efficacy, environmental fate and impact of imidacloprid under estuarine conditions. Additional trials are required in 2011 to improve

efficacy in tideflats that are silty or thickly vegetated, to obtain a more complete data set on the ecological impacts and persistence of imidacloprid under estuarine applications. Details on those trials are set forth in the attached document entitled "2012 study plan for imidacloprid in Willapa Bay." In this application we request an application window of May 1, 2012 to October 15, 2012.

Our applications of imidacloprid to limited acreage in Willapa Bay will not leach into ground water, nor will they have any opportunity to enter drinking water reservoirs. Imidacloprid from our treatments will quickly dissipate into the hundreds of thousands of gallons of moving waters within the estuary.

The Willapa Grays Harbor Oyster Growers Association (WGHOGA) submitted a preregistration package to EPA earlier in 2011. WGHOGA is working with the IR-4 Project to submit a Section 3 registration to the Agency in January of 2012. The request will be for 0.5 lbs a.i./acre rate of the 0.5% granular and the 2 pound flowable products.

#### **Label**

Restrictions on the proposed labels will include the application window (May 1– October 15), buffer zones for aerial and ground applications, and notification signs that better describe the nature and extent of the experimental treatments (see below).

"During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within quarter (1/4) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-fourth (1/4) of an inch high. Signs shall also include a map of the inlet that shows the treated area within a circle with a radius that extends 1/4 mile from the area's perimeter and the statement 'Do Not Fish, Crab, or Clam within 1/4 mile of area treated with experimental material, as indicated by the circle on the map.' Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment."

#### **Acreage and amount**

These trials will require a maximum of 45 lb a.i. of imidacloprid to be applied to a total of 90 total acres in Willapa Bay or Grays Harbor (25 lb a.i. of Nuprid 2F to 50 ac and 20 lb a.i. of Mallet 0.5G to 40 ac). However, depending on plot availability, the density and distribution of burrowing shrimp in 2012, and the treatment schedule for the conventional carbaryl-based management program for burrowing shrimp, the actual treated acreage could be considerably lower. The requested acreage is needed to complete the studies required for imidacloprid registration and permitting in the fifth of a multi-year experimental program. Amounts were derived according to an experimental design that strives for suitable replication but is constrained by limited space, time, and considerations for potential non-target impact. Our most common plot size (5 ac) tend to the low size of most commercial beds (10 ac) but are still large enough to



include some variation in burrowing shrimp density, substrate, vegetation, bed elevation, and drainage pattern that accompany commercial shellfish beds and impact efficacy.

**Duration**

We request that a federal experimental use permit for imidacloprid on Washington State shellfish grounds be granted for one year. The application window requested is between May 1, 2012 and October 15, 2012.

**Disposition of unused material**

Almost all imidacloprid will be used during experimental application, as the amount of material applied will be precisely measured and applied using calibrated equipment. Unused material will be stored temporarily in an EPA and OSHA compliant pesticide storage unit located at the Washington State University Research and Extension Unit in Long Beach, WA. Unused material will ultimately be disposed through the Washington Department of Agriculture's Pesticide Disposal Program.

The attachments and forms herein comprise the Application for an Experimental Use Permit to Ship and Use a Pesticide for Experimental Purposes Only (8570-17) with respect to imidacloprid to manage burrowing shrimp on Willapa Bay / Grays Harbor shellfish beds. The permit will allow us to continue tests of efficacy and non-target impact at a scale that more closely approximates commercial applications. These and subsequent tests will allow imidacloprid to advance toward registration and state permitting.

- A) **Additional information.** Chemical and Physical properties (see 2010 EUP application)
- B) Proposed label (see attached documents)
- C) Toxicity Data and Summary (see 2010 EUP application)
- D) Residue Data ( see 2010 EUP application)
- E) Effectiveness Data (see 2010 EUP application and attached 2011 progress report)
- F) Petition for Temporary Tolerance (see 2010 EUP application)
- G) Proposed Experimental Program (see attached 2011 imidacloprid report)

## 2012 STUDY PLAN FOR IMIDACLOPRID IN WILLAPA BAY

**TITLE:** Investigation of the use Imidacloprid for Burrowing Shrimp Management in Bivalve Aquaculture in Willapa Bay, Washington: 1) Impacts on Megafauna, Epibenthic and Benthic Organisms and 2) Persistence and Spatial Distribution of Imidacloprid within the Sediment Impact Zone

### PRINCIPAL INVESTIGATORS:

Dr. Kim Patten, Washington State University Long Beach Research and Extension Unit

Dr. Christian Grue, Washington Cooperative Fish and Wildlife Research Unit

School of Aquatic and Fishery Sciences, University of Washington

Dr. Steven Booth, Pacific Shellfish Institute

### OBJECTIVES

#### IMIDACLOPRID EFFICACY ASSESSMENTS

- Determine if post-treatment harrowing to destroy burrow integrity can be used to improve treatment efficacy on silty and vegetated sediment sites.

#### IMIDACLOPRID IMPACTS AND PERSISTENCE

- Measure concentrations of imidacloprid in water and the extents of its off-site movement.
- Measure concentrations of imidacloprid in eelgrass following treatment and assess the potential hazard to non-target organisms via its consumption.
- Measure the persistence of imidacloprid in sediment and the potential for natural recovery of the sediment impact zone, including field verification.
- Assess the magnitude, spatial extent and duration of impacts to benthic and epibenthic invertebrates following an application of imidacloprid to Willapa Bay tideflats.
- Measure the potential for natural recovery of the sediment impact zone, including field verification.
- Combine these elements into a comprehensive description of the sediment impact zone related to imidacloprid applications in Willapa Bay in general.

### METHODS

#### IMIDACLOPRID EFFICACY ASSESSMENTS

*Chemical treatment:* Mid-season applications (late May to July) of 0.5 lbs a.i./ac of liquid and granular formulations of imidacloprid will be made to 5 and 10 acre sandy sites infested with eelgrass and silty beds in Willapa Bay. The exact location and dimension of each plot has yet to be determined. Applications of imidacloprid will be made by air, boat and/or ATV. During the first or second tide following treatment, the sites will be dragged with a comb harrow from a barge to destroy the burrow integrity.

*Treatment assessment:* Treatment variables will be application timing, formulation, and harrowing timing, method and frequency. Efficacy at all sites will be assessed 2 weeks and 2



months post-treatment. Non-target impacts to megafauna will be assessed on and off-site, 24 and 48 hours following treatment. This includes the total number of Dungeness crabs with tetany and dead Dungeness crabs on and near each plot. Comparisons will be made with untreated sites and treated but non-harrowed sites within the plots.

#### IMIDACLOPRID IMPACTS AND PERSISTENCE

Research will be conducted with specific focus on the fate and transport of imidacloprid and its impact on the epibenthic and benthic infauna, with the ultimate goal of describing the sediment impact zone (SIZ) related to the potential commercial use of imidacloprid to manage burrowing shrimp in Willapa Bay and Grays Harbor. This research will be designed in conjunction with other activities related to registration and NPDES permitting of imidacloprid for use in these estuaries, particularly with regard to an Application for SIZ Authorization by Washington State Department of Ecology (Ecology). Accordingly, it will be prepared with input from that agency and under guidance of the Sediment Sampling and Analysis Plan Appendix (Ecology 2008) as well as Chapters 173-204 WAC, the Washington State Sediment Management Standards (Ecology 1995). The proposed plan is outlined below.

##### *Site Selection Parameters.*

The experimental design and sampling described below will allow sufficient comparisons of relevant parameters, descriptors, and other observations between or among: a) treated and untreated beds, b) beds treated with granular imidacloprid at 0.5 lb a.i./ac and flowable imidacloprid at 0.5 lb a.i./ac, and c) temporal differences. Due to constraints on resources and acreage available according to Federal and State Experiment Use Permits (EUP), the treatments will be 1) Nuprid 2F applied @ 0.5 lbs ai/ac to ~10 ac while fully exposed at low tide; 2) Mallet 0.5% G applied @ 0.5 lbs ai/ac to ~10 ac in shallow water as the tide retreats on partially sandy sediment; and 3) an untreated control site of ~ 10 ac. All sites will be similar in terms of sandy substrate, partial vegetation, density of burrowing shrimp, and bathymetry. Treated areas will be separated by at least 1000 m. Treatments will be applied by commercial aerial broadcast during a minus tide in July 2012. The sites proposed for these treatments are located west of Bay Center (Figure 1).

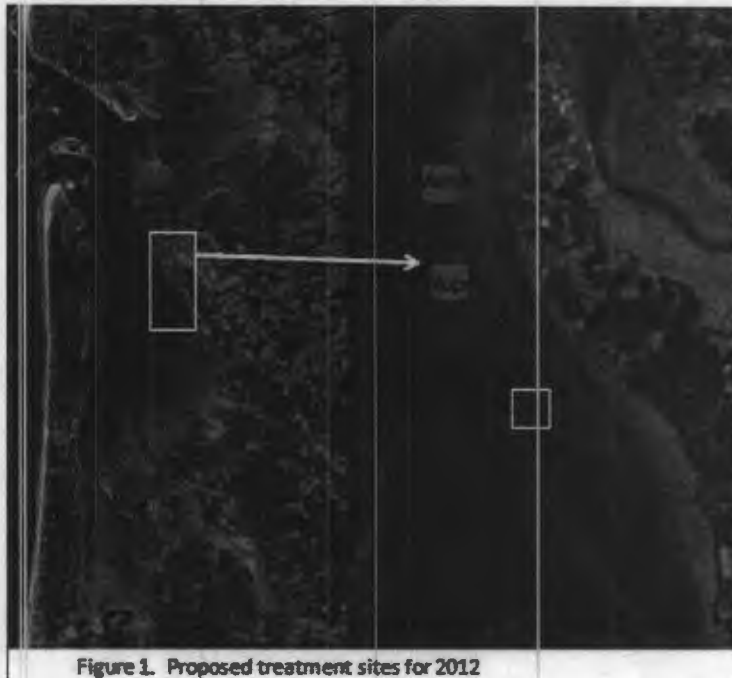


Figure 1. Proposed treatment sites for 2012

**Application Parameters:** Data collected on application will include application equipment and calibration protocol, temperature (air, water and sediment at 4 cm depth), wind (speed and direction), time and date of application, tide (time of low and magnitude of low tide and high tide), amount of water on plot at time of application, length of time prior to tidal inundation, percent cloud cover and solar radiation ( $\text{watts/m}^2$ ), direction of currents onto and off of treated area, location of on-bed tidal drainage channels, variation in beds elevations, sediment type, amount of vegetation coverage, rainfall, duration of application (start and stop time), history of burrowing shrimp treatment on the beds and in the area, and any treatment/application deviations.

**Sample location and replication.**

Water, sediment and vegetation samples will be collected at fixed sample points before and after treatment, using the timeline and locations matrix provided in Figure 1 and Table 1. Sample points will be chosen to maximize on-bed detection and off-bed movement. Off-bed sample points will be chosen by observing incoming and outgoing tide patterns and picking locations that funnel the highest volume of water over the treated area. Ebb flow tidal water will be sampled immediately after treatment for Nuprid and Mallet application.

Samples will be collected at the bed boundary and at distances from 30 to 240 m in the direction of tidal ebb flow. For both Mallet and Nuprid sites, the first incoming tidal water after treatment will be sampled at the bed center and near edge and extended out up to 240 m from the treatment area in the direction of water moving off the bed. A proposed mock-up of the location of these samples point is illustrated in Figure 1. Sample collection and analysis will follow an iterative process for vegetation, sediment and infauna. We will expand the sample collection across time and space to capture the potential for greater persistence in the sediment and greater spread of the



impact zone.

Analysis of samples will occur in increments expanding outward in space and time until three non-detects are reached. The exact specification of the iterative plan will be based on the pending results of data collected in 2011 and approval by Department of Ecology.

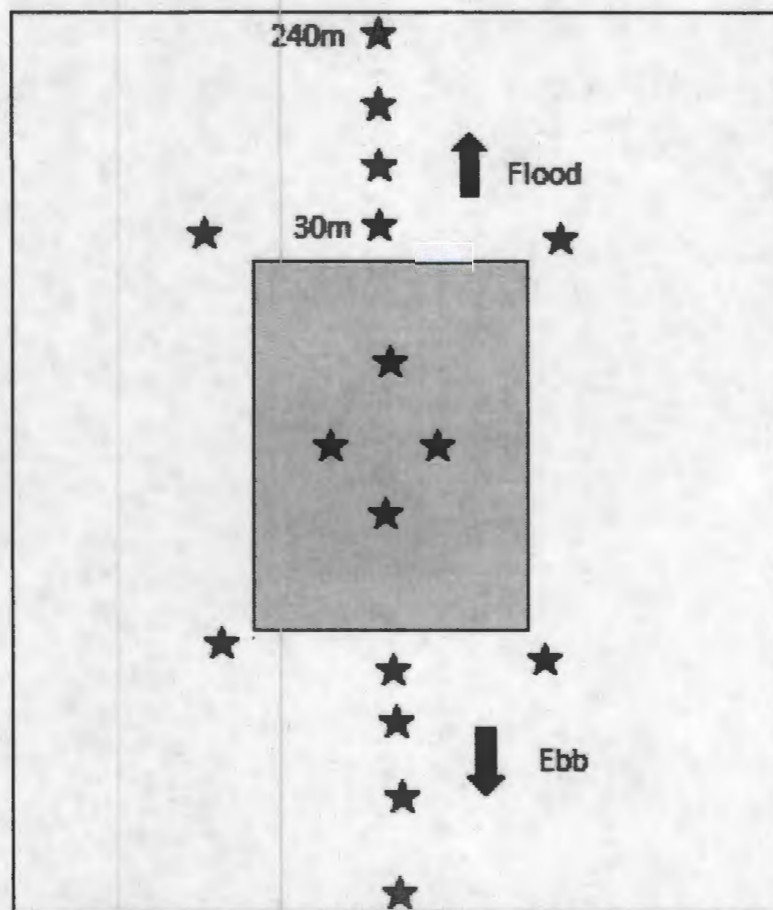


Figure 2. Proposed sample site locations.

**Table 1. Water, vegetation and sediment sampling plan for Willapa Bay imidacloprid treatments in 2012.**

site	sample location	water samples for imidacloprid analysis (hours after application)				vegetation samples for imidacloprid analysis (days after application)			
		0	2	24	54	1	4	14	28
		# of samples							
Mallet	on-bed	2	3	3	3	3	3	3	3
Mallet	off-bed ebb direction	6	3						
Mallet	off-bed flood direction		6	2	2	3	3	3	3
Nuprid	on-bed		3	3	3	3	3	3	3
Nuprid	off-bed ebb direction		6						
Nuprid	off-bed flood direction	3	6	2	2	3	3	3	3
control	on		1	1	1	2	2	2	2

site	sample location	sediment sample for imidacloprid analysis (days after treatment)								
		0	1	2	4	7	14	28	56	112
		# of samples								
Mallet	on-bed	4	4	4	4	4	4	4	4	4
Mallet	off-bed ebb direction	6	6	6	6	6	6	6	6	6
Mallet	off-bed flood direction	6	6	6	6	6	6	6	6	6
Nuprid	on-bed	4	4	4	4	4	4	4	4	4
Nuprid	off-bed ebb direction	6	6	6	6	6	6	6	6	6
Nuprid	off-bed flood direction	6	6	6	6	6	6	6	6	6
control	on	2	2	2	2	2	2	2	2	2

site	sample location	sediment samples for benthic infauna assessment (core replicates) (days after treatment)							
		0	1	2	4	14	28	56	70
		# of samples							
Mallet	on-bed	28	28	28	28	28	28	28	28
Mallet	off-bed flood direction	8	8	8	8	8	8	8	8
Nuprid	on-bed	28	28	28	28	28	28	28	28
Nuprid	off-bed ebb direction	8	8	8	8	8	8	8	8
control	on	28	28	28	28	28	28	28	28



### *Sample collection and analysis protocol.*

**Water.** Water samples will be collected in 1 L amber glass jars using a “clean hands/dirty hands” protocol. Collection times for each location are provided in Table 1. For ebb water samples collected at 0 HAT (immediately following treatment) samples will be collected in the middle of the water column until the jar is filled. Water depth at each of the ebb sampling points will be recorded since it is likely to vary considerable (5 to 50 cm) depending exact bathymetry of the point, tide and time. Incoming tidal water (on-bed and off-bed) will occur just as the tidal water flows over the sample point (~5-10 cm depth). This will provide worst case scenario for maximum water concentration.

Immediately after collection, water samples will be placed on ice and shipped on ice overnight to Pacific Agricultural Laboratory under chain of custody, where they will be stored at 1-4 C and analyzed within the EPA-recommended 7-day holding time. Imidacloprid analysis in water will be analyzed using the EPA 8321B (HPLC-MS QQQ) method to a reporting limit of 1.6 µg/l. Quality assurance will be by analysis of a method matrix blank and two matrix spike samples with expected percent recovery of 40 - 120%.

**Vegetation.** Eelgrass (*Zostera marina*) will be sampled from three on-bed sampling points in the middle of the bed and three off-bed sampling points, 30, 60, and 120 m from the edge of the plot in the main direction of water moving off-site. To assure the imidacloprid in the eelgrass samples is not from residual water or sediment on the vegetation, 1-liter samples will be collected in a 4-liter Zip-loc® bag using “clean hands-dirty hand” protocol. Samples will be placed on ice in a cooler and moved ~1000 m outside of the treatment zone and then gently triple rinsed with clean bay water to remove any surface sediment. Rinsed samples will be placed in 1 l Nalgene containers, in a dark-colored cooler on ice and shipped overnight on ice to Pacific Agricultural Laboratory, under chain of custody, where they will be analyzed for imidacloprid. Imidacloprid analysis for eelgrass water will be done by FDA PAM I 302 (HPLC-MS) method to a reporting limit of 0.010 mg/l, with quality assurance by analysis of a method matrix blank and two matrix spike samples with expected percent recovery of 40 - 120%.

**Sediment pore water.** At each time period and sample point, whole sediment will be sampled and placed in jars using a “clean hands/dirty hands” protocol with coring device designed to collect a sample 10 centimeters in depth. The coring device is a modified semi-transparent Nalgene® 500 ml HDPE bottle (7-cm diameter) with the bottom removed and hole drilled into the top shoulder of the bottle to create vacuum and allow extraction. Two cores will be collected at each sampling location and placed in 1 L Nalgene containers, in a dark-colored cooler on ice. Beginning with the initial sediment sample collection interval (0 hours post-application), the sediment core will be collected 1 meter to the north of the sample point.

On each successive sample collection interval (1, 2, 4, 7, 14, 28, 56, 112 days post-application), the exact point of sample collection will be rotated clockwise one cardinal direction such that the 1 day post sample was collected 1 meter east of the point, 24 hour post 1 meter south of the point and so on. This methodology will be employed to prevent previous sample collection efforts from interfering with subsequent sample collection. Samples will be transported off site in a plastic cooler with ice. Within 20 minutes, the samples will be moved to freezer storage at - 28

C. Once the samples are frozen they will be transported to the University of Washington and placed in temperature- monitored secure storage at - 40 C until time of analysis.

Sample analysis will be initiated by removal of sediment samples from frozen storage in 20-sample batches. Each batch will be allowed to defrost for approximately 12 hours. In an effort to bring each sample within the batch to the point of defrost at the same moment (as well as the interior and exterior of each sample simultaneously crossing the 0 C point), all samples will be placed in an insulated box to moderate the defrost cycle. Once slightly malleable (-2 to 0 C) each sample will be removed for homogenization. Once homogenized, approximately 400 grams of the sample will be removed and placed in a disposable sterile 500 ml Millipore Steritop® 0.22 micron filtration unit. Vacuum will be applied and the pore water extracted from the sample and collected into a clean 50 ml HDPE vial.

Analysis of imidacloprid concentrations within sediments will be conducted utilizing an enzyme-linked immunosorbent assay, ELISA. Additional information about the assay can be found at the manufacture's website ([envirologix.com](http://envirologix.com)). Every 20<sup>th</sup> analysis of sediment pore water by ELISA will be cross checked with an analysis using EPA 8321B (HPLC-MS QQQ) method. Should the ELISA method be found unsuitable for this study (based on data collected in 2010 and 2011 and discussion with Department of Ecology), then all analysis will be done by EPA 8321B (HPLC-MS QQQ).

Sediment epibenthic and benthic invertebrates. Epibenthic (crustaceans) and benthic (all other phyla) invertebrates will be sampled adjacent to the sediment sampling stations at both treated and untreated beds. Sample times will be pre-treatment, at 14, 28, 56, and 70 days after treatment, depending on results from the previous samples. These sample intervals will accurately describe impact to these highly mobile, highly reproductive, and mostly extremely abundant animals. A shorter post-treatment sample interval would be of little use, as our analyses cannot distinguish between animals alive or dead at the time of sample; absence is used as an indicator of death and benthic invertebrates degrade slowly in cold sediments.

Invertebrates will be sampled using a 10.2 cm internal diameter corer at 4 on-bed stations. Six replicate core samples will be collected per sample station for a total of 24 on-bed replicates at a 10 cm depth. An additional 4 replicates will be collected at one of the sample stations to a depth of 20 cm. Four additional core replicates will be taken at each of two off-bed stations sited at 60 m from the treated beds' boundaries in areas where the bed drains during ebb tide. Percent cover of *Z. japonica* or *Z. marina* will also be recorded at each sample location.

Each core will be immediately sieved through 0.5 mm mesh using salt water, then stored in a 10% buffered formalin solution and stained with rose bengal for 1-2 weeks, then re-sieved through 100 µm mesh to remove excess detritus and stored in 70% isopropyl alcohol. Species identification and enumeration will be done by Ruff Systematics (annelids) and PSI staff (crustaceans and mollusks) with most annelids and mollusks to species and crustaceans to family, order, or class.

The primary metric of comparison for treatment affect will be by direct comparison of absolute abundance, taxonomic richness, and Shannon-Wiener diversity of organisms within each of



Class Crustacea, Class Polychaeta, and Phylum Mollusca on beds treated with imidacloprid to untreated beds (reference or check beds), with an adverse affect determining when abundance or richness on treated is <50% of the mean values on the untreated bed. Statistical analyses will feature t-tests ( $\alpha=0.05$ ) between beds at each sample interval, so the duration of any impact can also be determined.

An additional analysis will feature comparisons of the change in the proportions of the primary descriptors on the treated bed between sample intervals. If the proportions do not change substantially or significantly after treatment, impact can be assumed to be minimal. If the proportions decline substantially after treatment, the impact can be assumed to be correspondingly greater. Note that a proportion of <33% is equivalent to the ratio of <50% that was used in the primary comparison, as described above. Change in the proportions of abundance, richness, and diversity provide a better assessment than change in their ratios on treated to untreated, as the latter sometimes involved dividing by zero, resulting in missing values and biasing the results. Proportions will be transformed to arcsine values prior to statistical analysis (t-test or one way analysis of variance ( $\alpha=0.05$ )).

Megafauna. Whole bed density of epibenthic megafauna (Dungeness crab and fish) will be assessed 24 hours after treatment at low tide by making close enough spaced transects (3 to 7 m) across each bed to allow counting of all affected (immobile) megafauna species on and within 50 m of the site. Data will be collected on individually size, species and type of impact (tetany, or death by any cause, directly or indirectly by tetany, e.g. bird predation of tetany crab). It is assumed that unaffected individuals will be mobile and move off-site prior to low tide.

Efficacy. Burrowing shrimp and polychaetes burrows will be counted 1 day before and 14 days after treatment on the treated sites and the control site. Ten 0.25 m<sup>2</sup> counts will be made at ten 5 x 5 m predetermined marked locations within each site. To test of off-site impacts, additional before and after counts will be made at locations 30 m and 60 m immediately outside the treatment zone in each cardinal direction.

#### *Project analysis.*

Because of the logistical demands of the field and analytical work, the full data array won't be forthcoming until late 2012/early 2013. Un-validated test results should be available by December 2012. Hypothesis testing will be done using parametric and nonparametric analyses. Other methods of analysis will be used as appropriate, including time-series. The final report for this project, along with the associated data submission, will be submitted to Department of Ecology and EPA within 12 months of treatment application.

# Material to be added to an e-Jacket/Jacket

Reg. No. 86414-EUP-1

Decision # \_\_\_\_\_

Description:

amendment - time extension, label  
revision

1. Placement within the e-Jacket/jacket:

☒ Default: (chronological, top = newest)

☐ File Location: (eg. "before page 45 in .pdf")  
\_\_\_\_\_

2. ☒ Send to Data Extraction contractors this material:

☒ Newly stamped accepted label

☐ Notification

☐ New CSF

☐ Other: \_\_\_\_\_

3. Attach this coversheet to the top of the material or jacket. It must be well organized and clipped together, NOT STAPLED. Then give the material with this coversheet to staff in the Information Services Center (Room S-4900).

Reviewer: Joanne Edwards

Phone: 305-6736

Division: RD

Date: 11/11/11





UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460-0001

OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

JAN 11 2011

Dr. Kim Patten  
Washington State University  
Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach, WA 98631

Subject: Amended Experimental Use Permit to Allow Use in 2011  
Experimental Use Permit No. 86414-EUP-1  
Effective Dates: Amended from "May 1, 2010 to October 31, 2010 to  
"April 15, 2011 to December 15, 2011"  
Quantity Authorized: Amended from "100 pounds of active ingredient" to  
"120 pounds of active ingredient"  
Your Application Dated December 13, 2010

Dear Dr. Patten;

There is no objection to the amended experimental use permit to allow use in 2011 under the terms listed above. A stamped copy of the label is enclosed for your records. This labeling must be used for all shipments of this product under the subject EUP. If you have questions, contact Joanne Edwards at (703) 305-6736 or by email at [edwards.joanne@epa.gov](mailto:edwards.joanne@epa.gov).

Sincerely yours,

A handwritten signature in black ink, appearing to read "John Hebert", is written over the typed name.

John Hebert, Product Manager 1  
Insecticide-Rodenticide Branch  
Registration Division (7504P)

# NUPRID 2F

## FOR EXPERIMENTAL USE ONLY

Experimental Use Permit Number: 86414-EUP-1

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

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**Permittee:**

Kim Patten, Extension Specialist, Professor  
Washington State University Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach WA 98631

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**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine . . . . . 21.4%

OTHER INGREDIENTS: . . . . . 78.6%

TOTAL: . . . . . 100.0%

Contains 2 pounds of imidacloprid per gallon.

## KEEP OUT OF REACH OF CHILDREN

### CAUTION – CAUCION

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No. 86414-EUP-1

**ACCEPTED**

*For shipment and use of product for experimental  
purposes under the provision of the Federal Insecticide,  
Fungicide, and Rodenticide Act, subject to attached  
comments.*

Permit No. 86414-EUP-1

Issued on January 6, 2010 and  
amended on January 11, 2011



FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>NOTE TO PHYSICIAN</b> No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS**  
**HAZARDS TO HUMANS AND DOMESTIC ANIMALS**  
**CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
  - Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
  - Shoes plus socks
  - Protective eyewear when working in a non-ventilated space
- Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Users must:**

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.

**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 2.0 lb a.i./ac using the following properly calibrated application equipment:

- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~ 20 gpa.
- SpikeWheel™ spoke wheel subsurface injectors operated from a floating platform at ~20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500 ft.
- With the exception of subsurface injections from a floating platform, all aerial and ground applications must be applied to beds exposed at low tide.
- All applications must occur between April 15 and December 15.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During applications, all public access areas within one-quarter (1/4) mile and all public boat launches within a one-and-a-half (1 1/2) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (1/4) of an inch high. Signs will include a map of the inlet that indicates the location of the treated area and an extended buffer that extends one-fourth (1/4) mile the area's perimeter and the statement "Do Not Fish, Crab, or Clam within 1/4 mile of area treated with experimental material, as indicated by the circle on the map". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.



## SPRAY DRIFT MANAGEMENT

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.

### Restrictions During Temperature Inversions

Because the potential for spray drift is high during temperature inversions, do NOT make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing, which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

### Importance of Droplet Size

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

## Mixing and Loading Requirements

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

## STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Refer to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk thorough spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak incidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.



Re: 2011 FEUP Application  
 Steven R. Booth  
 to:  
 Joanne Edwards  
 01/04/2011 11:29 AM  
 Show Details

Joanne,

I realized I forgot to shift signage from "aerial applications" to "all applicatiions".

The attached labels make that change.

Steve

----- Original Message -----

**From:** [Edwards.Joanne@epamail.epa.gov](mailto:Edwards.Joanne@epamail.epa.gov)  
**To:** [Steven R. Booth](#)  
**Sent:** Monday, January 03, 2011 8:41 AM  
**Subject:** Re: 2011 FEUP Application

I just looked at the EC label, and noted the following errors:

1. see your spacing problems on 1st page of label.
2. label needs to read April 15 (not 1)

also, I think you need to say something like-

with the exception of subsurface injections from a floating platform, all aerial and ground applications must be applied to beds exposed at low tide (or somethinglike that). (both labels)

The EC label also just says "During aerial application do not fish or crab within 1/4th mile of the treated area. I think you need to have this for all applications. Something we missed?

joanne

-----"Steven R. Booth" <boothswa@comcast.net> wrote: -----

**To:** Joanne Edwards/DC/USEPA/US@EPA  
**From:** "Steven R. Booth" <boothswa@comcast.net>  
**Date:** 01/03/2011 11:31AM  
**Subject:** Re: 2011 FEUP Application

Hi Joanne,

Happy New Year to you too.

The assumption (not proven yet) that the granular will not travel as far. Ground applications are also more precise. Also smaller, especially for these experimental trials. I had thought that the posting requirement was not on last year's granular label, but I see that it was.

Form Approved. OMB No. 2070-0040.



United States  
**ENVIRONMENTAL PROTECTION AGENCY**  
 Washington, DC 20460

OPP Identifier Number

Office of Pesticides Programs (7505C)

**Application for Experimental Use Permit to Ship and  
 Use a Pesticide for Experimental Purposes Only**

**1. Type of Application**

New



Amendment (See No. 2)



Extension (Give Permit Number below)

Permit Number

**2. Briefly explain (attach a separate sheet if necessary)**

This EUP is to be used to investigate the efficacy and nont-target effects of imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor, Washington.

**3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be issued (Include Zip Code) (Type or Print)**

Kim Patten, Extension Specialist, Professor  
 Washington State University Long Beach Research and Unit  
 2907 Pioneer Road  
 Long Beach WA 98631

**4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (Include Zip Code) (Type or Print)**

Nufarm Americas Inc.  
 150 Harvester Dr., Suite 200  
 Burr Ridge, IL 60527

EPA Company Number 81959-22

**5. Name of Product**

Name of registered product: Nuprid 2F

**6. Is Product Registered with EPA?**

No



Yes (Give Registration Number or File Symbol below)

Registration Number EPA Reg. No. 228-484

File Symbol

**7. Total Quantity of Product Proposed for Shipment/Use**

Pounds of formulated product 900

Pounds of active ingredient 120

**8. Acreage or Area to be Treated**

maximum 60 ac

**9. Proposed Period of Shipment/Use**

February 2011 – December 2011 (Ship)  
 April 15 2011 – December 15 2011 (Use)

**10. Places from which Shipped**

Nufarm Inland Empire Dist  
 1211E St Helens ST STE B, Pasco, WA 99301

**11. Crop/Site to be Treated**

Oysters and Manila Clams (Tapes philippinarum)  
 Willapa Bay and Grays Harbor, Washington

**12. Specify the name and number of the contact person most familiar with this application.**

Kim Patten 360-642-2031  
 Steven R. Booth 360-867-4163

**13. Signature of Applicant or Authorized Firm Representative**
**14. Title**

WGHOA IPM Coordinator

**15. Date Signed**

12/13/2010

**Certification**

This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.

I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.

**Below for EPA Use Only**

In any correspondence on this application, refer to this number

Normal review time indicates that processing of this application should be completed by (date)

Name of EPA Contact Person

Telephone Number

Received by:  
 EPA-OPP Registration Division  
 Washington, DC 20460



Re: eup1  
Steven R. Booth  
to:  
Joanne Edwards  
01/11/2011 06:41 PM  
Show Details

History: This message has been forwarded.

Thanks Joanne

----- Original Message -----

**From:** [Edwards.Joanne@epamail.epa.gov](mailto:Edwards.Joanne@epamail.epa.gov)  
**To:** [Steven R. Booth](#)  
**Sent:** Tuesday, January 11, 2011 3:34 PM  
**Subject:** Re: eup1

Steve- Email me a pdf of the revised application forms. I haven't sent the jackets for imaging yet, I just need to redo the letters and have John resign. I think it's ok. Joanne

-----"Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)> wrote: -----

**To:** Joanne [Edwards/DC/USEPA/US@EPA](mailto:Edwards/DC/USEPA/US@EPA)  
**From:** "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>  
**Date:** 01/11/2011 05:19PM  
**Subject:** Re: eup1

Thanks for checking Joanne,

For last year, I have 40 lb ai authorized for the 0.5G and 100 lb ai authorized for the 2F.

For this year, we requested 30 lb ai for the 0.5G and 180 lb ai for the 2F (Dec 13 submission), so we can treat 60 ac with the 0.5G at 0.5 lb ai/ac and 90 ac with the 2F at 2lb ai/ac, but we can live with 60 ac of the 2F.

So my request is:

For Experimental Use Permit No. 86414-EUP-1 (submitted Dec 13, 2010 and authorized Jan 11, 2011), we request to amend the amount authorized from 90 lb active ingredient to 120 lb active ingredient.

Thanks again for all your help on this Joanne.

Steve

----- Original Message -----

**From:** [Edwards.Joanne@epamail.epa.gov](mailto:Edwards.Joanne@epamail.epa.gov)  
**To:** [Steven R. Booth](#)  
**Sent:** Tuesday, January 11, 2011 11:32 AM  
**Subject:** RE: eup1

no I don't think so. This is an EUP, for testing efficacy purposes only. But I can check with John. Email the amounts you want to increase from (from XX to XX). I'll check with John tomorrow.

-----"Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)> wrote: -----

To: Joanne [Edwards/DC/USEPA/US@EPA](mailto:Edwards/DC/USEPA/US@EPA)  
 From: "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>  
 Date: 01/11/2011 01:03PM  
 Subject: RE: eup1

Can it be amended to the higher amount?

-----Original Message-----

From: Edwards.Joanne@epamail.epa.gov [<mailto:Edwards.Joanne@epamail.epa.gov>]

Sent: Tuesday, January 11, 2011 9:43 AM

To: Steven R. Booth

Subject: RE: eup1

Steve- Look at your original submission. That's where I got the amounts from. We are authorizing about what you asked for last year. This is an experimental use permit.

Joanne Edwards  
 EPA/OPPTS/OPP/RD/IRB  
 (703) 305-6736  
[edwards.joanne@epa.gov](mailto:edwards.joanne@epa.gov)

From: "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>

To: Joanne Edwards/DC/USEPA/US@EPA

Date: 01/11/2011 12:36 PM

Subject: RE: eup1

Joanne,



I thought we asked for 90 ac of the 2F and maybe 50 ac of the 0.5G, but I am not sure if I have the final 1850-17 forms on this computer. The last date I have on this computer is Dec 17 where I asked for 180 lb ai for the 2F which would go to 90 ac.

-----Original Message-----

From: Edwards.Joanne@epamail.epa.gov [<mailto:Edwards.Joanne@epamail.epa.gov>]

Sent: Tuesday, January 11, 2011 8:12 AM

To: Steven R. Booth

Subject: Fw: eup1

the other one

Joanne Edwards

EPA/OPPTS/OPP/RD/IRB

(703) 305-6736

edwards.joanne@epa.gov

----- Forwarded by Joanne Edwards/DC/USEPA/US on 01/11/2011 11:11 AM

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From: cts/cts/QP/USEPA/US@EPA

To: Joanne Edwards/DC/USEPA/US@EPA

Date: 01/11/2011 10:43 AM

Subject: eup1

Please open the attached document. This document was digitally sent to you using an HP Digital Sending device. (See attached file: [Untitled].pdf)

Do you want me to put it back on?

Steve

----- Original Message -----

**From:** [Edwards.Joanne@epamail.epa.gov](mailto:Edwards.Joanne@epamail.epa.gov)

**To:** [Steven R. Booth](#)

**Sent:** Monday, January 03, 2011 8:09 AM

**Subject:** RE: 2011 FEUP Application

Hi Steve Happy New Year. back to work. Going through all the emails. Looked over your revised application.

You redid the language on notification signs. Raised a question. During aerial application do not fish or crab within 1/4th mile of the treated area. Shouldn't you also have this for ground applications too?

joanne

-----"Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)> wrote: -----

**To:** Joanne Edwards/DC/USEPA/US@EPA, John Hebert/DC/USEPA/US@EPA

**From:** "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>

**Date:** 12/17/2010 02:31PM

**Cc:** "Kim Patten" <[pattenk@wsu.edu](mailto:pattenk@wsu.edu)>

**Subject:** RE: 2011 FEUP Application

Joanne,

Attached are:

- 1 corrected the 8570-17 forms (I just spaced on the acreage for the Nuprid the other day – forgot it was the 2F) by submitting as “extension” and providing permit number.
- 2 Experimental labels formatted according to previous labels with changes in restrictions related only to a) extended treatment window, b) clarification of area treated in notification signs (include map on signs)
- 3 Attachment 1 with reference to above changes in restrictions from previous years
- 4 Attachment II drops reference to temporary tolerance (that was included in previous applications but I guess it is not relevant to these EUPs.



In a previous email (below) you noted "It (the application) needs to come in through previous channels in order to be processed". I am not sure what that means – I have previously submitted through you.

I am taking the afternoon off, but will make any other changes or processes if needed on Monday.

Have a good weekend,

Steve

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**From:** Edwards.Joanne@epamail.epa.gov [mailto:Edwards.Joanne@epamail.epa.gov]  
**Sent:** Thursday, December 16, 2010 12:07 PM  
**To:** Steven R. Booth; Hebert.John@epamail.epa.gov  
**Subject:** RE: 2011 FEUP Application

John needs to weigh in on this, but I see no reason why we can't just "re-extend"..

Then the only thing you would need to do is to resubmit the labels (to like just like what we've already approved) and redo the application form.

I do have a pre-registration package, and did route it for review to EFED. I guess Alan S. will request a meeting early next year. Joanne

-----"Steven R. Booth" <boothswa@comcast.net> wrote: -----

To: Joanne Edwards/DC/USEPA/US@EPA, John Hebert/DC/USEPA/US@EPA  
 From: "Steven R. Booth" <boothswa@comcast.net>  
 Date: 12/16/2010 02:38PM  
 Cc: "Kim Patten" <pattenk@wsu.edu>  
 Subject: RE: 2011 FEUP Application

Thanks Joanne,

I will take care of these items.

However, I can comment on a couple of them now.

I thought this was a "new" application because we have been running on annual EUPs, but I just checked last year's submittal and that was indeed an "extension".

I worked off the labels for our draft proposed labels for final registered product (Protector), but will go back to last year's labels for the EUP.

In the restrictions sections, we did want to expand the treatment window a bit more than last year's permitted window and perhaps decrease the buffers to main channels a bit, but the latter is not critical.

Thank you for your quick response,

Steve

-----Original Message-----

From: Edwards.Joanne@epamail.epa.gov [<mailto:Edwards.Joanne@epamail.epa.gov>]

Sent: Thursday, December 16, 2010 9:35 AM

To: Steven R. Booth; Hebert.John@epamail.epa.gov

Cc: Steven R. Booth; Kim Patten

Subject: Re: 2011 FEUP Application

Hi Steve- I printed out what you submitted and looked over. It needs to come in through normal channels in order to be processed.

I have the following comments:

shouldn't this be an extension (see box 1 on the application form)

What exactly are you doing different? The labels you submitted are missing information (First AID etc.) You need to take the labels we approved last year and resubmit them, Two copies, one which is highlighted in the areas that you have changed. You shouldn't be changing anything in the RESTRICTIONS. And under directions for use, you need the language "To test for efficacy..."

Your application (8570-17) makes no sense. Box number 9 just talks about shipping of material.. This box must have the dates of use. This is an EUP, not a federal registration.

For the liquid product, you have almost doubled the amount of material to be used. But the acreage remains at 90. This makes no sense. The application rate and acrea amount have to add up in the math. This is an experimental use, not a federal registration, so there are limits to what you can apply

You also need to redo pg 45 of your application, where you talk about PETITION FOR TEMPORARY TOLERANCE. The oysters can't be eaten. This is experimental use only.

Joanne Edwards



EPA/OPPTS/OPP/RD/IRB  
 (703) 305-6736  
 edwards.joanne@epa.gov

From: "Steven R. Booth" <boothswa@comcast.net>

To: Joanne Edwards/DC/USEPA/US@EPA

Cc: "Kim Patten" <pattenk@wsu.edu>, "Steven R. Booth" <boothswa@comcast.net>

Date: 12/14/2010 08:57 PM

Subject: 2011 FEUP Application

Hi Joanne,

Attached is the Willapa Grays Harbor Oyster Growers application packet for a Federal Experimental Use Permit to apply imidacloprid on Willapa Bay tidelands in 2011. These include the 8570-17 forms for both Nuprid 2F and Mallet 0.5G, their experimental labels, and Attachments I and II. I have also attached our proposed experimental labels for the flowable and granular products we hope to register soon.

As in previous years, Washington State University (Dr. Kim Patten) is the official submitter.

Please let me know if you need any more information or clarification.

Sincerely,

Steve Booth[attachment "WGHOGA Attachments I & II.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "Mallet 0.5G Exp Label Dec 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "Nuprid 2F Exp Label Dec 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "WGHOGA 8570-17 Mallet Dec 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "WGHOGA 8570-17 Nuprid Dec 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "Proposed Federal 2F Label.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "Proposed Federal 0.5G Label.pdf" deleted by Joanne Edwards/DC/USEPA/US]

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[attachment "WGHOGA FEUP Attachments 1 &2 Dec 17 2010.pdf" removed by Joanne Edwards/DC/USEPA/US]

[attachment "MALLET EXP LABEL 86414-Dec 17 2010.pdf" removed by Joanne Edwards/DC/USEPA/US]

[attachment "NUPRID EXP LABEL 86414-EUP DEC 17 2010.pdf" removed by Joanne Edwards/DC/USEPA/US]

[attachment "WGHOGA 8570-17 Mallet Dec 17 2010.pdf" removed by Joanne Edwards/DC/USEPA/US]

[attachment "WGHOGA 8570-17 Nuprid Dec 17 2010.pdf" removed by Joanne Edwards/DC/USEPA/US]



Form Approved. OMB No. 2070-0040.



United States  
**ENVIRONMENTAL PROTECTION AGENCY**  
 Washington, DC 20460

OPP Identifier Number

Office of Pesticides Programs (7505C)

**Application for Experimental Use Permit to Ship and  
 Use a Pesticide for Experimental Purposes Only**

**1. Type of Application**
☐

New

☐

Amendment (See No. 2)

☒

Extension (Give Permit Number below)

Permit Number 86414-EUP-1

**2. Briefly explain (attach a separate sheet if necessary)**

This EUP is to be used to investigate the efficacy and nont-target effects of imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor, Washington.

**3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be issued (Include Zip Code) (Type or Print)**

Kim Patten, Extension Specialist, Professor  
 Washington State University Long Beach Research and Unit  
 2907 Pioneer Road  
 Long Beach WA 98631

**4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (Include Zip Code) (Type or Print)**

Nufarm Americas Inc.  
 150 Harvester Dr., Suite 200  
 Burr Ridge, IL 60527

EPA Company Number 81959-22

**5. Name of Product**

Name of registered product: Nuprid 2F

**6. Is Product Registered with EPA?**
☐

No

☒

Yes (Give Registration Number or File Symbol below)

Registration Number EPA Reg. No. 228-484

File Symbol

**7. Total Quantity of Product Proposed for Shipment/Use**

Pounds of formulated product 450

Pounds of active ingredient 90

**8. Acreage or Area to be Treated**

maximum 90 ac

**9. Proposed Period of Shipment/Use**

February 2011 -- December 2011 (Ship)  
 April 15 2011 -- December 15 2011 (Use)

**10. Places from which Shipped**

Nufarm Inland Empire Dist  
 1211E St Helens ST STE B, Pasco, WA 99301

**11. Crop/Site to be Treated**

Oysters and Manila Clams (Tapes philippinarum)  
 Willapa Bay and Grays Harbor, Washington

**12. Specify the name and number of the contact person most familiar with this application.**

Kim Patten 360-642-2031  
 Steven R. Booth 360-867-4163

**13. Signature of Applicant or Authorized Firm Representative**
**14. Title**

WGHOA IPM Coordinator

**15. Date Signed**

12/13/2010

**Certification**

This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.

I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.

**Below for EPA Use Only**

In any correspondence on this application, refer to this number

Received by:  
 EPA-OPP Registration Division,  
 Washington, DC 20460

Normal review time indicates that processing of this application should be completed by (date)

Name of EPA Contact Person

Telephone Number



**NUPRID 2F**

**FOR EXPERIMENTAL USE ONLY**

Experimental Use Permit Number: 86414-EUP-1

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

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**Permittee:**

**Kim Patten, Extension Specialist, Professor  
Washington State University Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach WA 98631**

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**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine . . . . .21.4%

OTHER INGREDIENTS: . . . . .78.6%

TOTAL: . . . . .100.0%

Contains 2 pounds of imidacloprid per gallon.

**KEEP OUT OF REACH OF CHILDREN**

**CAUTION – CAUCION**

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No. 86414-EUP-1

FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>NOTE TO PHYSICIAN</b> No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS  
HAZARDS TO HUMANS AND DOMESTIC ANIMALS  
CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
  - Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
  - Shoes plus socks
  - Protective eyewear when working in a non-ventilated space
- Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Users must:**

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.

**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 2.0 lb a.i./ac using the following properly calibrated application equipment:

- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~20 gpa.
- SpikeWheel™ spoke wheel subsurface injectors operated from a floating platform at ~20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500 ft.
- For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.
- All applications must occur between April 1 and December 15.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within a one-and-a-half (1 1/2) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (1/4) of an inch high. Signs will include a map of the inlet that indicates the location of the treated area and an extended buffer that extends one-fourth (1/4) mile the area's perimeter and the statement "Do Not Fish, Crab, or Clam within 1/4 mile of area treated with experimental material, as indicated by the circle on the map". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

**SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind



speed. Do not apply when winds are greater than 10 mph or during temperature inversions.

#### Restrictions During Temperature Inversions

Because the potential for spray drift is high during temperature inversions, do NOT make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing, which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are

#### STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Refer to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk through spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak incidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.

and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

#### Importance of Droplet Size

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

#### Mixing and Loading Requirements

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

**ATTACHMENT 1 – Explanation and Justification**

Two indigenous species of burrowing shrimp severely impact both the mudflat community and oyster production in Willapa Bay and Grays Harbor, WA. Both ghost shrimp (*Neotrypaea californiensis*) and mud shrimp (*Upogebia pugettensis*) reside in burrows beneath the mudflat surface, where they abrogate habitat from other benthic organisms and severely disrupt the structure of the mudflat substrate by bioturbation, causing cultured and native bivalves to sink and die. Although indigenous, both species, but particularly ghost shrimp, have greatly increased in density and distribution in the last 60 years, likely due to a combination of factors including loss of seasonal freshwater influx since the damming of the Columbia River and a decrease in key predators due to over-fishing.

Since the 1960s, applications of carbaryl (Sevin® 80SP, Bayer Corp.) on selected and legally limited acreage of commercial oyster beds, have effectively suppressed burrowing shrimp. A single application usually sufficed through multiple years of oyster development. A suite of best management practices, such as seasonal placement of carbaryl to avoid migratory salmon and pre-season monitoring of target beds, ensured that the estuarine ecosystem was not significantly affected. However, the potential impact of many conventional (i.e., organophosphate and carbamate) pesticides has been questioned by a variety of groups. This was most recently demonstrated by the National Marine Fisheries Biological Opinion regarding the impact of three carbamate pesticides on Pacific Endangered Salmon.

Without the ability to manage burrowing shrimp, a significant portion of the local shellfish industry would no longer be economically viable. In 1990, oyster aquaculture accounted for one of every twelve jobs in Pacific County. Since then, the decline in marine fisheries has made the local economy even more dependent on shellfish production. As demonstrated elsewhere, the collapse of agricultural and other resource-based industries often leads to increased private development and pollution.

Efforts by the Willapa Bay / Grays Harbor Oyster Growers Association (WGHOGA) to develop an IPM program have been ongoing since the inception of the carbaryl-based program, but were formalized in 2001 when a memorandum of agreement was signed with several organizations and state agencies to develop an IPM program. Investigations of alternatives to carbaryl currently involves dozens of scientists, extension agents, and grower-collaborators who focus on biological, mechanical, and chemical controls, as well as a better understanding of burrowing shrimp ecology. Some biological control options show potential for implementation in the future, but will require much more research. Some reduced risk compounds partially suppress burrowing shrimp populations, but densities remain above farmable levels. At this point, we have identified only a single alternative tactic, imidacloprid, that has sufficient efficacy, environmental compatibility, and potential for registration to control burrowing shrimp and allow shellfish farming to continue in Southwest Washington beyond 2012.

Although preliminary very small plot trials of imidacloprid (Admire 2EC @ 0.5 lb a.i./ac) showed efficacy comparable to carbaryl (Sevin WP or SP @ 10 lb a.i./ac), the results of large scale trials in 2008 were disappointing (see Effectiveness Data, Figure 14, Attachment 2). An application of granular imidacloprid (Mallet 0.5G), applied at 0.5 lb a.i./ac to a 9 ac plot in 2009 also showed limited efficacy. Results of small plot trials of both materials in 2009 and in early



spring 2010 showed efficacy of the two different formulations was inconsistent and likely depends on such factors as type of substrate, bed elevation, and amount of vegetation (Tables 24, 25). These factors vary throughout the bay, requiring treatment of larger acreage to accurately determine best use of the materials. Large scale trials in 2010 (Table 36) generally supported the importance of these factors, but more trials are needed to fully describe their importance.

The seasonal timing of application is another related factor that likely contributes to the efficacy of imidacloprid against burrowing shrimp. Applications in April and May were more effective in small plot trials in 2009 and 2010, likely due to the relatively lower density of eelgrass and absence of algal mats that often develop in late June and July. The 2010 FEUP allowed applications from May to October, but we were unable to test the importance of early season applications in large plots in 2010 due to restrictions in our NPDES permit, which we intend to rectify for 2011. In this application, we request an application window of April 15 – December 15, 2011. That change is indicated in one of the restrictions in the experimental labels for Nuprid 2F and Mallet 0.5G.

Another restriction includes a more detailed description of nature and extent of the experimental treatments that will be included on the notification signs that will be posted near the treatment sites<sup>1</sup>. The previous restriction regarding notification signs provided little information regarding the size of the closure.

The trials will also test different application methods. The liquid formulation can easily be applied via the conventional methods for the standard carbaryl-based program: either aerially using helicopters or ground-based sprayer systems. The 0.05% active ingredient in the granular material makes the formulation extremely heavy, complicating application. Application by boat may be simpler than on bare ground yet also improve efficacy, as floating vegetation would allow greater penetration of the substrate surface.

Several studies of non-target impact and fate & transport of imidacloprid in the water column and in sediments are required for the registration of permitting of its use on shellfish beds. While some of these studies have been and continue to be addressed in the laboratory, they also need to be assessed and validated in the field under commercial situations. Data will be gathered this summer to address these studies.

Our applications of imidacloprid to limited acreage in Willapa Bay will not leach into ground water, nor will it have any opportunity to enter drinking water reservoirs. Imidacloprid from our treatments will quickly dissipate into the hundreds of thousands of gallons of moving waters within the estuary.

A pre-registration packet recently was submitted to EPA that comprised updates on studies required for registration, a request for waiver of the aquatic metabolism study, and proposed labels for the eventual registration of the granular and flowable formulations of imidacloprid (Protector 0.5G and Protector 2F, respectively). Restrictions on the proposed labels include a reference to the new proposed window (April 15 - December 15) and changes to the notification signs, as described above, as well as easements of the buffer zones from 200 ft and 50 ft to 100 ft and 50 ft for aerial and ground applications of Protector 2F, respectively. Buffers for Protector 0.5G are proposed to remain at 100 ft for aerial applications but be reduced to 25 ft

for ground applications, respectively.

These attachments, experimental labels (Nuprid 2F and Mallet 0.5G) and forms (8570-17) comprise the Application for an Experimental Use Permit to Ship and Use a Pesticide for Experimental Purposes Only with respect to imidacloprid to manage burrowing shrimp on Willapa Bay / Grays Harbor shellfish beds. The permit will allow us to continue tests of efficacy and non-target impact at a scale that more closely approximates commercial applications. These and subsequent tests will allow imidacloprid to advance toward registration and state permitting.

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<sup>1</sup> "During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within quarter (1/4) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-fourth (1/4) of an inch high. Signs will include a map of the inlet that indicates the location of the treated area and an extended buffer that extends one-fourth (1/4) mile the area's perimeter and the statement "Do Not Fish, Crab, or Clam within 1/4 mile of area treated with experimental material, as indicated by the circle on the map". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment."



**ATTACHMENT 2****A) Chemical and Physical Properties**

- 1) Chemical names: 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine.
- 2) Molecular formula:  $C_9H_{10}ClN_5O_2$
- 3) Tradename: Imida E-AG 2 F (EPA Reg. No. 81959-22)
- 4) Formulation (2 lbs active ingredient per gallon) of imidacloprid
- 5) CAS Number: 13826-41-3
- 6) Molecular Weight: 255.7
- 7) Water Solubility: 0.51 g/l (200° C)
- 8) Solubility in Other Solvents: @ 20° C
  - a) dichloromethane - 50.0 - 100.0 g/l
  - b) isopropanol - 1.0-2.0 g/l
  - c) toluene - 0.5-1.0 g/l
  - d) n-hexane - <0.1 g/l
  - e) fat - 0.061 g/100g
- 9) Melting Point: 136.4-143.8° C., 143.8° C (crystal form 1) 136.4° C (crystal form 2)
- 10) Vapor Pressure: 0.2 uPa (20° C) ( $1.5 \times 10^{-9}$  mmHg)
- 11) Partition Coefficient: 0.57 (22° C). (Kidd, H. and James, D. R., Eds. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, 1991 (As Updated).10-2)
- 12) Adsorption Coefficient:
  - a) in a low organic carbon silt loam (0.9% OC),  $K_d = 2.4$  mL/g (Oi, M. 1999. Time-dependent sorption of imidacloprid in two different soils. J. Agric. Food Chem. 47: 327-332.13).
  - b) see Table 1. (Felsot and Rupert, 2002).

Table 1. Sediment Distribution Coefficients ( $K_d$ ) and Freundlich Sorption Coefficient ( $K_f$ ) for Imidacloprid in Willapa Bay Sediments and Sediments Mixed with Activated Carbon.

Initial solution concn, mg/L	sediment distribution coefficient ( $K_d$ , mgL/g)		
	CaCl <sub>2</sub>	saltwater	saltwater carbon/sediment (1:2)
0.01	0.59	0.52	3912
0.1	0.62	0.52	824
1	0.51	0.45	785
10	0.39	0.32	766
100	0.28	0.24	763
av $K_d$	0.48	0.41	1410
SD	0.14	0.13	1399
$K_f$	0.46	0.40	520
1/n	0.91	0.91	0.86

**B) Proposed Label**

See separate documents

**C) Toxicity Data and Summary** [1-7 mostly from ETOXNET (<http://extoxnet.orst.edu/pips/imidaclo.htm>)]

- 1) Acute toxicity
  - a) ORL-RAT: LD<sub>50</sub> 450 mg kg<sup>-1</sup> (Meister 1994)
  - b) ORL-MUS: LD<sub>50</sub> 131 mg kg<sup>-1</sup> (Kidd and James 1991)
  - c) 24-hour DML-RAT: >5,000 mg/kg.
  - d) Non-irritating to eyes and skin (rabbits), and non-sensitizing to skin (guinea pigs) (Kidd and James 1991)
- 2) Chronic Toxicity
  - a) A 2-year feeding study in rats fed up to 1,800 ppm resulted in a No Observable Effect Level (NOEL) of 100 ppm (5.7 mg/kg body weight in males and 7.6 mg/kg in females). Adverse effects included decreased body weight gain in females at 300 ppm, and increased thyroid lesions in males at 300 ppm and females at 900 ppm.
  - b) A 1-year feeding study in dogs fed up to 2,500 ppm resulted in a NOEL of 1,250 ppm (41 mg/kg). Adverse effects included increased cholesterol levels in the blood, and some stress to the liver (measured by elevated liver cytochrome p-450 levels) (Federal Register 1995).
- 3) Reproductive Effects
  - a) A three generation reproduction study in rats fed up to 700 ppm imidacloprid resulted in a NOEL of 100 ppm (equivalent to 8 mg/kg/day) based on decreased pup body weight observed at the 250 ppm dose level (Federal Register 1995).
- 4) Teratogenic Effects
  - a) A developmental toxicity study in rats given doses up to 100 ppm by gavage on days 6 to 16 of gestation resulted in a NOEL of 30 mg/kg/day (based on skeletal abnormalities observed at the next highest dose tested of 100 ppm) (Federal Register 1995)
  - b) In a developmental toxicity study with rabbits given doses of imidacloprid by gavage during days 6 through 19 of gestation, resulted in a NOEL of 24 mg/kg/day based on decreased body weight and skeletal abnormalities observed at 72 mg/kg/day (highest dose tested) (Pike et al. 1994).
- 5) Mutagenic Effects
  - a) Imidacloprid may be weakly mutagenic. In a battery of 23 laboratory mutagenicity assays, imidacloprid tested negative for mutagenic effects in all but two of the assays. It did test positive for causing changes in chromosomes in human lymphocytes, as well as testing positive for genotoxicity in Chinese hamster ovary cells (Pike et al. 1994).
- 6) Carcinogenic Effects
  - a) Imidacloprid is considered to be of minimal carcinogenic risk, and is thus categorized by EPA as a "Group E" carcinogen (evidence of noncarcinogenicity for humans). There were no carcinogenic effects in a 2-year carcinogenicity study in rats fed up to 1,800 ppm imidacloprid (Anatra-Cordone and Durkin 2005).
- 7) Organ Toxicity
  - a) In short-term feeding studies in rats, there were thyroid lesions associated with very high doses of imidacloprid (Pike et al. 1994).
- 8) Fate in Humans and Animals
  - a) Imidacloprid is quickly and almost completely absorbed from the gastrointestinal tract, and eliminated via urine and feces (70-80% and 20-30%, respectively, of the 96% of the parent compound administered within 48 hours). The most important metabolic steps include the degradation to 6-chloronicotinic acid, a compound that acts on the nervous system as described above. This compound may be conjugated with glycine and eliminated, or reduced to guanidine (USEPA 1995).



## 9) Toxicity to Aquatic Organisms

## a) Fish

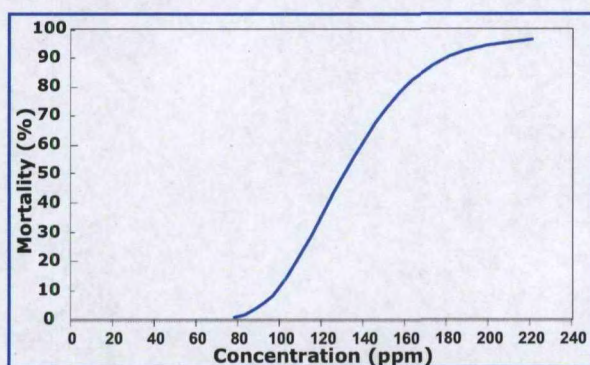
## (1) Dose-response

- (a) bluegill (fresh): static 96-hr acute  $LC_{50}$ , >105 mg a.i./L (Bowman and Bucksath 1990a)
- (b) rainbow trout (fresh), chinook smolts (salt), sheepshead minnow (salt) (Table 2)
- (c) chinook smolts (Figure 1)
- (d) "Using the standard classification scheme proposed by U.S. EPA/EFED (2001), imidacloprid would be classified as practically nontoxic to fish."  
(Anatra-Cordone and Durkin, 2005. Section 4.1.3.1, p 412)

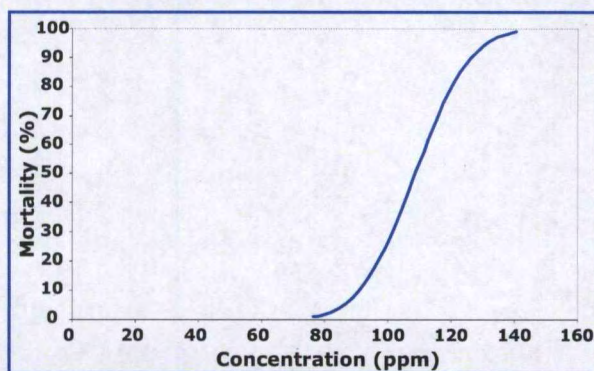
Table 2. Toxicity of imidacloprid to fish (as presented in Anatra-Cordone and Durkin 2005, Appendix 5, except for †, C. Grue, unpublished data 2007)

Species	Exposure	Effects	Reference
<b>FRESHWATER Acute Toxicity:</b>			
Rainbow Trout ( <i>Ochorhynchus mykiss</i> ) mean length 5.3 cm, mean weight 1.3 g, 10 per concentration	Static 96-hour acute toxicity study with technical grade NTN 33893 (95.3% a.i.). Nominal concentrations of 0, 50, 89, 158, 281, 500 mg a.i./L, with measured greater than 80% of nominal values	48-hr $EC_{50}$ = 85 mg/L, 95% CI = 71 - 113 mg/L 48-hr NOAEC (immobility) = 42 mg/L Mobility was the endpoint of assessment	Young and Hicks 1990 MRID 42055317
Rainbow Trout † ( <i>Ochorhynchus mykiss</i> ) mean weight 0.3 g, 10 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Admire 2F (21.4% a.i.) Nominal concentrations of 0, 15, 22, 32, 46, 66, 96, 139, 202 mg a.i./L	96-hr $LC_{50}$ = 170 mg/L, 95% CI = 159 - 181 mg/L 96-hr NOAEC (lethargy) = 22 mg a.i./L (14% at 96 hr)	Grue and Frew unpublished data
Rainbow Trout † ( <i>Ochorhynchus mykiss</i> ) mean weight 23 g, 7 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Admire 2F (21.4% a.i.) Nominal concentrations of 0, 75, 107, 151, 215, 305 mg a.i./L	96-hr $LC_{50}$ = 163 mg/L, 95% CI = 148 - 177 mg/L 96-hr NOAEC (lethargy) = < 75 mg a.i./L	Grue and Frew unpublished data
White sturgeon † ( <i>Acipenser transmontanus</i> ) juvenile, mean weight 28 g 5 per concentration	Static 96-hour acute toxicity study with Nuprid 2F (21.4% a.i.) Nominal concentrations of 0, 46, 66, 96, 139, 202, 294 mg a.i./L measured concentrations at: T0 h: 50, 100, and 220 mg a.i./L for nominal of 46, 96 and 202 mg a.i./L; T96 h: 50, 100, and 220 mg a.i./L	96-hr $LC_{50}$ = 124 mg/L, 95% CI = 93 - 170 mg/L 96-hr NOAEC (lethargy) = 66 mg a.i./L (Figure 1)	Grue and Frew unpublished data
<b>FRESHWATER Chronic Toxicity:</b>			
Rainbow Trout ( <i>Ochorhynchus mykiss</i> ), newly fertilized eggs <4 hours old, 4 replicates of 35 eggs each per concentration, plus an additional 50 eggs per each of the 4 control replicates (egg viability determination)	98-Day flow-through early life stage test with technical grade NTN 33893 at nominal concentrations of 0, 1.3, 2.5, 5.0, 10 and 20 mg/L equivalent to mean measured concentrations of 0, 1.2, 2.3, 4.9, 9.8 and 19 mg/L	<u>original conclusions:</u> NOAEC = 9.8 mg/L LOAEC = 19 mg/L (statistically significant reduction in length at 36 and 60 days post-hatch, and body weight at 60 days posthatch). No statistically significant biologically important effects on egg viability, hatch, survival or behavioral variables were observed. MATC (maximum acceptable toxicant concentration) = 14 mg/L (geometric mean of NOAEC and LOAEC)	Cohle and Bucksath 1991 MRID 42055320

		<u>1992 re-evaluation:</u> Day 36 growth was most sensitive endpoint. Based on reevaluation of this endpoint: NOAEC = 1.2 mg a.i./L LOAEC = 2.3 mg a.i./L MATC = 1.7 mg a.i./L	Gagliano 1992 MRID 42466501
<b>SALTWATER Acute Toxicity:</b>			
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), young adult, mean length 29 mm, mean weight 0.77 g, 10 per concentration	Static 96-hour acute toxicity test of technical grade NTN 33893 (96.2% a.i.). Control, solvent control, 22.4, 35.2, 58.2, 105 and 195 mg/L mean measured concentrations	96-hour $LC_{50}$ = 161 mg a.i./L, 95% CI = 105 - infinity, NOAEC = 58.2 mg a.i./L on the basis of mortality and signs (lethargy, dark coloration) at higher concentrations.	Ward 1990a MRID 42055318
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), 4-day old, 10 per replicate, 4 replicates per concentration 24-h static renewal	Static 96-hour acute toxicity test of Imida EAG2F (21.4% a.i.) Nominal concentrations of 0, 10, 20, 40, 80, 160 mg a.i./L, mean measured concentrations to verify serial dilutions: 10, 78, and 150 mg a.i./L	96-hr $LC_{50}$ = 61 mg/L, 95% CI = 50-70 mg/L 96-hr NOAEC (lethargy) = 40 mg a.i./L	Frew, Grue and Curran, 2007 unpublished data
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), fertilized eggs, 15 per replicate, 4 replicates per concentration ≥ 80% hatch	32-day early life stage toxicity test (USEPA OPPTS 850.1400) of Imida E AG 2F (21.4% a.i.) Nominal concentrations of 0, 0.625, 1.25, 2.5, 5, and 10 mg a.i./L mean measured concentrations to verify serial dilutions: 0.59, 2.3, 9.5 mg a.i./L.	No adverse effects on survival or growth at any concentration tested. NOAEC = 10 mg a.i./L	Curran, Frew and Grue 2008, unpublished report, Nautilus Environmental
Chinook Salmon † ( <i>Ochorhynchus tshawitsha</i> ) mean weight 7 g, 10 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Imida 2F (21.4% a.i.) Nominal concentrations of 0, 46, 66, 96, 139, 202, 294 mg a.i./L	96-hr $LC_{50}$ = 109 mg/L (figure 2), 95% CI = 102 - 118 mg/L 96-hr NOAEC (lethargy) = 66 mg a.i./L (Figure 2)	Grue and Frew unpublished data



**Figure 1** Dose-response curve for White sturgeon juveniles exposed to Nuprid 2F in freshwater for 96 hr.  $LC_{50}$  = 124 mg a.i./L, CI = 93 – 170 mg a.i./L. C. Grue unpublished data



**Figure 2** Dose-response curve for Chinook smolts (7g) exposed to Imida 2F in seawater for 96 hr.  $LC_{50}$  = 109 mg a.i./L, CI = 102 – 118 mg a.i./L. C. Grue, unpublished data



(2) Local (Willapa) Field Tests (Table 3; Patten et al., 2007)

(3) Local (Willapa) Lab Tests (Table 4; Patten et al., 2008)

Saddleback gunnel collected in Willapa Bay and maintained in aquaria for 5 days prior to testing. 5 fish per replicate, 3 replicates per concentration. Fish exposed to imidacloprid in estuarine water (56 – 64° F) in 1 L jars.

Table 3. Effects of carbaryl (Sevin) and imidacloprid (Imida) overspray on fish in tide pools.

Treatment	% survival at 48 hr after treatment	
	staghorn sculpin	threespine stickleback
Sevin 80SP (8 lb a.i./ac)	11.3 <i>b</i>	64.0 <i>b</i>
Imida 2F (0.5 lb a.i./ac)	100.0	100.0
untreated check	100.0	100.0

\* means followed by the same letter are not significantly different (Duncans Multiple Range; P=0.05).

Table 4. Effects of imidacloprid concentration and exposure time on survival of saddleback gunnel (*Pholis ornata*).

Concentration (ppm)	% Survival			
	4 hr	24 hr	48	96 hr
0	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>
10	100.0	100.0	100.0	100.0
100	100.0	100.0	100.0	93.3

\* means followed by the same letter are not significantly different (LSD; P=0.05). *n.s.*, not significant

## Relevant Aquatic Invertebrates (Freshwater Insects not included)

(4) Dose Response Parameter (Table 5).

From Anatra-Cordone and Durkin, 2005: "Amphipod crustaceans such as *Hyaella azteca*, the saltwater Mysid, *Mysidopsis bahia*, and the fresh water insect midge, *Chironomus tentans*, are the most sensitive species. In freshwater, the water flea, *Daphnia magna*, was the least sensitive species, while in saltwater, the eastern oyster as least sensitive. Acute toxicity values range from a 96-hour NOAEC of 0.000035 mg/L for *H. azteca* (England and Bucksath 1991), to a 96-hour NOAEC of 145 mg/L for eastern oyster (Wheat and Ward 1991). On the basis of longer-term studies designed to assess reproduction, growth and survival, *M. bahia* was the most sensitive species, with an NOAEC value of 0.000163 mg a.i. imidacloprid/L for growth and reproductive success (Ward 1991), and *D. magna* was the most tolerant species with a 21-day NOAEC for immobility of 1.8 mg/L (Young and Blake 1990)."

Table 5. Toxicity of imidacloprid to relevant aquatic invertebrates (mostly as presented in Anatra-Cordone and Durkin, 2005; Appendix 6).

Species	Exposure	Effects	Reference
<b>FRESHWATER Acute Toxicity:</b>			
Water flea ( <i>Daphnia magna</i> ), 2 flasks per concentration with 10 each	Static 48-hour acute toxicity study with NTN 33893 (95.9% a.i.) at nominal concentrations up to 125 mg/L with actual mean concentrations of 0, 15, 25, 42, 71 and 113 mg/L	96-hour LC50: 211 mg a.i./L (158 - 281 mg a.i./L). 96-hour NOAEC: 50 mg a.i./L 89 mg/L and higher: apathy, irregular swimming behavior, lying on side/back, staggering 281 mg/L and higher: mortality	Grau 1988a MRID 42055316 Ward 1990a MRID 42055318
<i>Hyaella azteca</i> (amphipod crustacean), 2-3 mm juveniles, 2 replicates per concentration, 10 per replicate	Static acute toxicity test with NTN 33893 at measured concentrations of control, 0.00035, 0.00097, 0.0035, 0.010, 0.034, 0.100, 0.340, 1.000 and 3.100 mg/L	96-hr LC50: 0.526 mg/L, 95% CI = 0.194 - 1.263 mg/L 96-hr EC50 (immobilization): 0.055 mg/L, 95% CI = 0.034 - 0.093 mg/L 96-hr NOAEC (immobilization and abnormal effects, such as lethargy or surfacing) = 0.00035 mg/L	England and Bucksath 1991 MRID 42256303

<i>Hyalella azteca</i> (amphipod crustacean), 14 - 21 days old, 2 replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33823 metabolite at mean measured concentrations of 0, 5.6, 11.0, 22.1, 43.8 and 86.8 mg/L	96-hour LC50: 51.8 mg a.i./L, 95% CI = 44.0 - 60.9 mg a.i./L 96-hour EC50 (immobilization): 29.0 mg a.i./L, 95% CI = 24.7 - 34.0 mg a.i./L 96-hour NOAEC (mortality): 22.1 mg a.i./L	Rooney and Bowers 1996 MRID 43946601
<i>Hyalella azteca</i> (amphipod crustacean), 7 - 21 days old, 2 replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33519 urea metabolite at nominal (measured) concentrations of 0, 6.25 (5.81), 12.5 (11.80), 25 (23.46), 50 (46.80), and 100 (94.83) mg a.i./L	96-hour LC50: > 94.83 mg a.i./L, 96-hour EC50 (immobilization): > 94.83 mg a.i./L, 96-hour NOAEC: 94.83 mg a.i./L	Dobbs and Frank 1996a MRID 43946603
<b>FRESHWATER Chronic Toxicity:</b>			
Water flea ( <i>Daphnia magna</i> ), 4 replicate jars per concentration, 6 1 <sup>st</sup> instar daphnids per jar	Chronic static renewal toxicity study of technical grade NTN 33893. Control, solvent control, 0.46, 0.86, 1.8, 3.6, and 7.3 mg/L	21-day EC50 (immobilization): >7.3 mg/L MATC = 2.5 mg/L (1.8 - 3.6 mg/L) NOAEC = 1.8 mg/L LOAEC = 3.6 mg/L 3.6 and 7.3 mg/L: Significantly reduced adult daphnid length in comparison with pooled controls 7.3 mg/L: Significantly reduced survival; significantly reduced mean young/adult reproduction days in comparison with pooled controls. No effects on time to first brood at any concentration.	Young and Blake 1990 MRID 42055321
<b>SALTWATER Acute Toxicity:</b>			
<i>Artemia</i> sp., and Mosquito ( <i>Aedes taeniorhynchus</i> ) 3 trials, 4 replicates per concentration, 10 animals each species per replicate	Static 48-hr acute toxicity test. Technical grade imidacloprid (>95% purity)	<u>Artemia:</u> 48-hr LC50 = 361.23 mg/L, 95% CI = 307.83 - 498.09 mg/L <u>Mosquito:</u> 48-hr LC50 = 0.13 mg/L, 95% CI = 0.010 - 0.016 mg/L Note: increasing salinity increased sensitivity to imidacloprid	Song et al 1997; Song and Brown 1998
Mysid ( <i>Mysidopsis bahia</i> ), < 24 hours old, 10 per concentration.	96-hr flow-through acute toxicity tests of technical grade NTN 33893 (96.2% a.i.). Mean measured concentrations: 1 <sup>st</sup> test: control, solvent control, 0.032, 0.0584, 0.0937, 0.146 and 0.249 mg a.i./L 2 <sup>nd</sup> test: control, solvent control, 0.00842, 0.0133, 0.0229, 0.0372 and 0.0634 mg a.i./L	<u>First test:</u> 96-hr LC50 = 0.0377 mg a.i./L, 95% CI = 0.0267 - 0.0464 mg a.i./L, NOAEC not determined. <u>Second test:</u> 96-hr LC50 = 0.0341 mg a.i./L, 95% CI = 0.0229 - 0.0372 mg a.i./L, NOAEC = 0.0133 mg a.i./L on the basis of mortality and loss of equilibrium at higher doses.	Ward 1990b MRID 42055319
Mysid ( <i>Mysidopsis bahia</i> ), < 24 hours old, 2 replicates per concentration, 10 per replicate	96-Hr flow-through acute toxicity test, NTN 33893 240 FS Formulation, control, solvent control, 18 (21), 29 (31), 49 (56), 82 (78), 136 (125) and 227 (219) ug a.i./L nominal (measured) concentrations	96-hr LC50 = 0.036 mg a.i./L, 95% CI = 0.031 - 0.042 mg a.i./L NOAEC (mortality) = 0.021 mg a.i./L	Lintott 1992 MRID 42528301



Eastern Oyster ( <i>Crassostrea virginica</i> ), 20 per concentration	96-hr flow-through test of effect on shell growth. Technical grade NTN 33893 (95.8% and 96.2% a.i. for 2 <sup>nd</sup> and 1 <sup>st</sup> tests, respectively) 1 <sup>st</sup> test: control, solvent control, 2.93, 5.14, 8.19, 14.2, and 23.3 mg a.i./L, measured 2 <sup>nd</sup> test: control, 145.0 mg a.i./L, measured	<u>First test:</u> 100% survival; No effects on new shell growth <u>Second test:</u> 100% survival; new shell growth of exposed was 22% less than controls. This was statistically significant. 96-hr NOAEC: 145 mg/L	Wheat and Ward 1991 MRID 42256305
<b>SALTWATER Chronic Toxicity:</b>			
Midge ( <i>Chironomus tentans</i> ), second instar, 2 replicates per concentration, 10 chironomids per replicate	Static renewal 96-hr toxicity test with technical grade NTN 33893 (95.0 % a.i.) control, solvent control, measured concentrations of 0.00067, 0.00124, 0.00339, 0.0102, 0.0345, 0.100, and 0.329 mg a.i./L	10-day LC50: 0.00317 mg/L, 95% CI = 0.00124 - 0.0102 mg/L 10-day survival NOAEC: 0.00124 mg/L 10-day growth NOAEC: 0.00067 mg/L (basis = dry weight of survivors)	Gagliano 1991 MRID 42256304
Mysid ( <i>Mysidopsis bahia</i> ), <24- hrs old, 4 replicates per concentration, 15 mysids per replicate cup	Flow-through chronic toxicity tests with technical grade NTN 33893 (96.2% a.i.) <u>First test:</u> control, solvent control, 560, 1290, 2850, 5080 and 10100 ng a.i./L mean measured <u>Second test:</u> control, solvent control, 36.8, 78.4, 163, 326 and 643 ng a.i./L nominal	<u>First Test:</u> <u>1290 ng/L and higher:</u> Significantly reduced number of offspring per female reproductive day <u>5080 ng/L and higher:</u> significantly reduced growth of 1 <sup>st</sup> generation mysids as total length and dry weight <u>10,100 ng/L:</u> Statistically increased mortality in comparison with pooled controls for first generation. No effects on mortality in 2 <sup>nd</sup> generation <u>MATC (reproductive success):</u> 849 ng/L (560 - 1290 ng/L) <u>MATC (growth):</u> 3806 ng/L (2850-5080 ng/L) <u>Second Test:</u> No effects on number of offspring per female reproductive day. <u>326 and 643 ng/L:</u> Significantly reduced growth of 1 <sup>st</sup> generation as total length and dry weight in comparison with pooled controls <u>643 ng/L:</u> Statistically increased mortality in comparison with pooled controls for 1 <sup>st</sup> generation. No effects on mortality in 2 <sup>nd</sup> generation. <u>MATC (reproductive success):</u> > 643 ng/L <u>MATC (growth):</u> 230 ng/L (163 - 3260 ng/L) No real explanation for discrepancy between 1 <sup>st</sup> and 2 <sup>nd</sup> tests with regard to growth.	Ward, 1991 MRID 42055322

## (5) Local (Willapa) Tests

## i) Diploid oyster larvae

## (a) Survival (Table 5)

All tests featured diploid Pacific oyster larvae from  
Taylor Shellfish within 2 weeks of test. No of  
individuals per replicate and type of arena as

Table 5. Effects of imidacloprid on survival of diploid Pacific oyster larvae following 24 hr exposure in 3 arenas.

Arena	Sample Size	Concentration (ppm)	% Survival *
test-tube	15 - 20	0	67.2 n.s.
		1	69.7
		5	47.1
		10	30.7
		20	41.6



specified. 3 replicates per concentration. Tests in water bath at 79 – 80°F for 24 hr. Oysters identified as live or dead based on swimming activity.

Percent survival was not significantly different from plain estuarine water at less than 50 ppm imidacloprid. (Patten unpublished data, 2008)

(b) Survival set, growth (Table 6)

As above, except 4 replicates per concentration; 3 oyster shells per 1 L glass jar. Survival measured after 24 hr exposure and shells transferred to growout bags in Willapa Bay, 6 inches above the tidal substrate, at -1.0 tide height. Number of set oysters and diameter measured after 158 days growout.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

ii) Set, growth of triploid oyster larvae (Table 7)

As above, except triploid Pacific oyster larvae obtained from Taylor Shellfish within 2 weeks of testing, 4 shells per replicate / jar, diameter measured after 172 days in growout bags after 24 hr exposure to imidacloprid.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

iii) Growth of diploid Pacific juvenile oysters (Table 8)

As above, except 5 small juvenile ( $\bar{x}$  surface area = 8.5 mm<sup>2</sup>) diploid Pacific oysters per shell, 3 shells per replicate, 3 replicates per concentration, exposed to imidacloprid in fresh estuarine water for 96 hr, then transferred to growout bags for 158 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

iv) Growth of diploid juvenile oysters (Table 9)

As above, except initial juvenile diploid Pacific oyster length was 7.8 mm, 6 oysters per replicate, 3 replicates per treatment, growout for 273 days.

Impact was not significantly different from estuarine water at any concentration or variable (Patten unpublished data, 2008)

250 ml cups	30 – 40	0	15.7 <i>b</i>
		1	10.0 <i>b</i>
		10	18.0 <i>b</i>
		100	0 <i>a</i>
1 L jars	10 – 25	0	48.0 <i>n.s.</i>
		1	28.0
		10	69.0
		20	23.0
1 L jars	30 – 70	0	38.0 <i>b</i>
		5	6.0 <i>b</i>
		50	0 <i>a</i>
		500	0 <i>a</i>

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 6. Effects of imidacloprid on survival, set, and development (diameter) of diploid Pacific oyster larvae after 24 hr exposure.

Sample Size	Concentration (ppm)	% Survival*	No. Set	Diameter (mm)
100 – 150	0	54.5 <i>n.s.</i>	9.3 <i>n.s.</i>	7.8 <i>n.s.</i>
	10	42.0	15.8	8.8
	100	33.0	14.8	8.7
	1000	42.7	18.0	8.6

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 7. Effects of imidacloprid on set and development (diameter) of triploid Pacific oyster larvae following 96 hr exposure.

Sample Size	Concentration (ppm)	No. Set	Diameter (mm)
14 – 150	0	2.4 <i>n.s.</i>	21.9 <i>n.s.</i>
	5	1.3	26.3
	50	1.1	28.1

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 8. Effects of 96 hr exposure to imidacloprid on development of diploid juvenile oysters after 158 days growout.

Concentration (ppm)	Surface Area (mm <sup>2</sup> )
0	8639 <i>n.s.</i>
10	10071
100	9306
1000	7797

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 9. Effects of imidacloprid at 48 and 96 hr exposures on length of juvenile (7.8 mm length) oysters after 273 days growout.

Concentration (ppm)	Length (mm)	
	48	96
0	54 <i>n.s.</i>	48 <i>n.s.</i>
10	53	42
100	37	46
1000	59	39

\* means followed by the same letter are not significantly different (LSD; P=0.05).



v) Growth of juvenile Kumomoto oysters  
(Table 10)

As above, except 5 small juvenile ( $\bar{x}$  diameter = 18 mm<sup>2</sup>)

Kumomoto oysters from Taylor Shellfish per replicate, 3 replicates per concentration, exposed to imidacloprid in fresh estuarine water for 48 or 96 hr, then transferred to growout bags for 92 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

Table 10. Effects of imidacloprid on development (diameter) of juvenile Kumomoto oysters after 24 or 96 hr exposure and 92 days growout.

Concentration (ppm)	Diameter (mm <sup>2</sup> )	
	24 hr	96 hr
0	28.2 n.s.	27.9 n.s.
10	23.4	26.3
100	25.5	27.3

\* means followed by the same letter are not significantly different (LSD; P=0.05).

vi) Manila clams

(a) Preliminary tests by size  
(Figure 3)

Water temperatures for 3 – 6 mm clams, 67° F, others, 48 – 49°F. Survival rates were > 50% for all size classes at imidacloprid concentrations < 1000 ppm (Patten, unpublished data, 2007)

(b) Small clams, (Table 11)

Methods as above for 2008 lab tests, except ~120 small ( $\bar{x}$  diameter = 4.75 mm) Manila clams per replicate / 1 L jar, 5 replicates per concentration. Clams were triple rinsed after treatment then placed on sieved sand. Mortality assessed as not burrowing in sand after 24 hr. Live clams placed in 1 mm mesh growout bags for 30 days, then transferred to 2 mm mesh bags for 46 days. Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

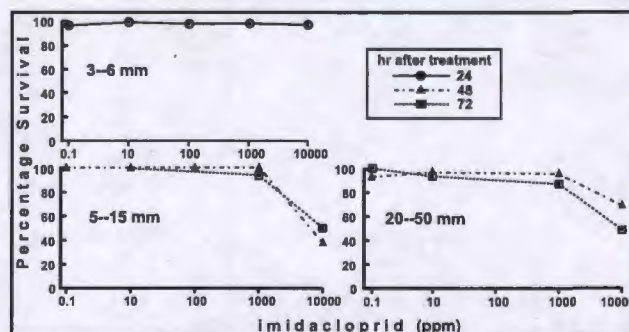


Figure 3 Effects of imidacloprid (Admire 4.6F) on Manila clams of different size classes.

Table 11. Effects of imidacloprid on survival of juvenile Manila clams at exposure intervals and development (diameter) after 76 days growout.

Exposure Interval	Concentration (ppm)	% Survival	Diameter (mm)
48	0	91.7 n.s.	6.0 n.s.
	1	94.5	6.4
	10	90.8	6.9
	100	87.8	5.4
96	0	93.3 n.s.	6.8 n.s.
	1	92.5	7.7
	10	90.2	7.0
	100	91.1	5.9

\* means followed by the same letter are not significantly different (LSD; P=0.05).

vii) Dungeness crab megalopae

(a) Preliminary 2008 lab trials

Collected as megalopae using light trap on June 16, 2008, but most metamorphosed to first post-larval instar during exposure to imidacloprid 7 days later. Single individual per replicate, 3 replicates per concentration, 3 exposure intervals per concentration. No mortality at any treatment combination of 0, 10, 100 ppm imidacloprid and 4, 24, 48, and 96 hr exposure intervals. (Patten unpublished data, 2008)

(b) 2009 lab trials (Table 12, Figure 4).

Crab were collected as megalopae over three nights in late May and maintained in aerated seawater until testing on May 27. Megalopae were exposed to 0, 0.5, 1, 5, 10 ppm concentrations of imidacloprid for 4 hours and 18 hours. Two sets of 10 megalopae per each concentration/exposure interval were treated in 10 ml containers, then transferred to four 1L containers (i.e., 5 megalopae per replicate) of aerated estuarine water where they were monitored daily for 7 days.



## Crab megalopae exposed to

imidacloprid at  $\leq 5$  ppm for 4 hr showed temporary (24 hrs) tetany, but all molted to first instar juveniles. Megalopae exposed to  $\leq 1$  ppm for 18 hrs all molted.

Mortality was generally greater at higher concentrations, longer exposure time, and increasing hours after exposure, but mortality at low concentrations sometimes confounded probit analysis. The probit model significantly fit 4 of the 9 data sets (Table 12) and showed lower  $LC_{50}$  values at the longer exposure time (Figure 4).

Table 12. Chi-square statistics for Pearson Goodness of Fit to probit model to larval and first instar crab exposed to imidacloprid concentrations for 4 or 18 hr exposure intervals (EI) and at 5 post application intervals (hours after treatment (HAT)).

EI	HAT	Chi-square	df <sup>a</sup>	sig <sup>b</sup>
4	156	29.416	14	<b>.009</b>
	108	31.171	14	<b>.005</b>
	36	8.657	14	.852
	26	NC*		
	4	NC		
18	152	11.235	14	.665
	104	23.602	14	<b>.051</b>
	32	34.688	14	<b>.002</b>
	22	NC		

<sup>a</sup> Degrees of freedom (4 replicates at 4 doses; 5 individuals per replicate)

<sup>b</sup> Significance level; Models with levels  $< 0.15$  are significant (in bold)

\* NC, Not Computed; the ratios of response counts to subject counts are the same, i.e. the slope is zero

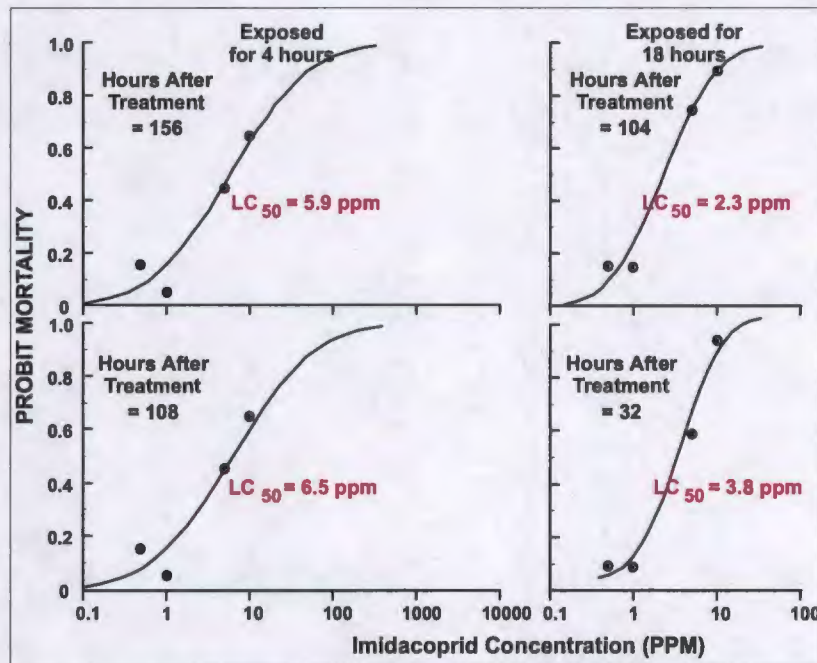


Figure 4 Concentration/response probit curves of 1<sup>st</sup> instar Dungeness crab exposed to imidacloprid for 4 and 18 hr at 4 post-application intervals.

## viii) Juvenile Dungeness Crab

## (a) Initial 2007 lab trials (Table 13)

Mortality was very low in juvenile crab (carapace width  $< 3$ " ) exposed to 0.5 lb a.i./ac imidacloprid in the field, but larger crab showed substantial tetanus shock in large scale field trials (see below). (Patten unpublished data, 2007)

Table 13. Two tests of carbaryl (Sevin 80SP) and imidacloprid (Imida 2F) overspray on juvenile Dungeness crab in tide pools.

Treatment	Days After Treatment	% Mortality*
Sevin 80SP (8 lb	14	70 <i>b</i>
Imida 2F (0.5 lb a.i./ac)	14	0.21 <i>a</i>
untreated check	14	0
Imida 2F (5.0 lb a.i./ac)	21	90 <i>a</i>
untreated check	21	86 <i>a</i>

\* means followed by the same letter are not significantly different (Duncans Multiple Range;  $P=0.05$ ).



## (b) 2009 lab trials (Table 14)

Young of the year (YOY) Dungeness crab ranging in size from 12 -- 44 mm carapace width were collected from Willapa Bay in July 2009 and immediately placed in aerated 5L aquaria filled with 3L fresh bay water and 1L clean ocean beach sand. After crab were settled and buried, each container was treated with 0, 1, 2 or 4 lb ai/ac equivalent of 0.5% granular formulation of imidacloprid (Mallet 0.5G). Water temperature was 14 -- 16° C. After 24 hr, crab were removed, rinsed in bay water and placed in an aquaria filled with fresh bay water and sand. Three replicate aquaria with 8 -- 10 crab each comprised each treatment rate. Crab were gently teased from the sand and observed for mobility or mortality at 24, 96 and 144 hours after the 24 hr exposure. Crab were fed diced razor clams during the course of the study.

After 24 hours exposure to Mallet0.5G at rates up to 4 lbs ai/ac equivalent, YOY Dungeness crab showed no significant indications of tetany or mortality (Patten unpublished data, 2009).

(c) 2009 field trials  
(Table 15)

YOY Dungeness crab (12 -- 44 mm

carapace width) were collected in July 2009 in Willapa Bay, confined in ¼" wire mesh, open bottom, screen cages that were 6" in diameter and 12" tall. Cages were buried to a depth of 4" in tideflats near Nahcotta, WA in Willapa Bay. The site contained thick Japanese eelgrass (*Zostera japonica*) growing in sand. After the crab had burrowed into the sand, the sites were treated in separate isolated 28' by 28' plots with Etigra 2F at 2 or 4 lb ai/ac or Mallet 0.5% G at 0.5 or 1 lb ai/ac. Treatments were replicated among 3 cage per each formulation/rate combination and 4 crab per cage. Cages were treated at low tide with a 3 hr interval before inundation by the incoming tide. Crabs were assessed for mortality at 4 and 7 days after treatment by opening each cage and gently sifting them from the sand.

Percentage mortality was low and did not differ significantly at either post application interval (ANOVA) (Patten unpublished data, 2009).

(d) 2010 lab trials (Tables 16,  
17, Figure 5)

Young of the year (YOY) Dungeness crab were collected in June 2010 in Willapa Bay. Size varied from 9 -- 18 mm carapace width and 0.3 -- 1.25 g/crab. Following collection, six crab of mixed size classes were placed in 1L containers filled with ½L of 0, 0.065, 0.125, 0.25, 0.5, 0.75, 1, 1.5 and 3 ppm concentrations of imidacloprid in bay water. Each treatment rate was replicated among 4 aerated containers. After 4 hr exposure, crab were rinsed in fresh bay water and transferred to new containers and ½L fresh bay water. Number of mobile crab were counted at 1, 2, 3, 4, 19, and 86 hr after the 4 hr exposure. Water was replaced every 48 hours. Water temperature was 14 -- 16° C. Crab were fed fresh bay cockles after 86 hours. Data were analyzed by ANOVA and by probit analysis.

Two to four hours exposure of YOY Dungeness crab to imidacloprid at rates > 1.5 ppm resulted in substantial loss of mobility, but the loss was often reversed after 19 hr, even at an exposure concentration of 6 ppm (Table 16). The probit model significantly fit 3 of the 6 data sets (Table 17) and LC<sub>50</sub> values were 3.7, 1.8, 1.7 ppm at 2, 3, and 4 hours after treatment, respectively (Figure 5) (Patten unpublished data 2009).

Table 14. Mean percentage mobile YOY Dungeness crab after 24 hr exposure to 4 concentrations of imidacloprid (Mallet 0.5G) plus no concentration at 3 post exposure intervals (Hours After Exposure).

Exposure Concentration (lb ai/ac equivalent)	Hours After Exposure		
	24	48	96
0	100	100	96.7
0.5	100	100	91.3
1	93.3	93.3	86.7
2	93.3	93.3	96.7
4	96.7	86.7	86.7

Table 15. Mean percentage mortality of YOY Dungeness crab caged and treated in the field with granular (Mallet 0.5G) and liquid (Nuprid 2F) formulated imidacloprid at two post-application intervals (Days After Treatment).

Formulation	Rate (lb ai/ac)	Days After Treatment	
		4	7
Mallet 0.5G	0.5	16.7	11.1
	1.0	11.1	22.2
Nuprid 2F	2.0	11.1	55.6
	4.0	0.0	11.1

Table 16. Chi-square statistics for Pearson Goodness of Fit to probit model to YOY crab exposed to imidacloprid concentrations for 4 hr exposure interval (EI) and at 6 post application intervals (hours after treatment (HAT)).

EI	HAT	Chi-square	df <sup>a</sup>	sig <sup>b</sup>
4	86	7.568	7	.372
	19	8.147	7	.320
	4	37.098	7	<b>.000</b>
	3	20.604	7	<b>.004</b>
	2	17.603	7	<b>.014</b>
	1	6.164	7	.521

<sup>a</sup> Degrees of freedom (4 replicates at 4 doses; 5 individuals per replicate)  
<sup>b</sup> Significance level; Models with levels < 0.15 are significant (in bold)

Table 14. Mean percentage mobile YOY crabs after short-term exposure (4 hr) to 9 concentrations of imidacloprid plus no concentration at 6 post exposure intervals (Hours After Exposure).

Exposure concentration (ppm)	Hours After Exposure					
	1	2	3	4	19	86
0	92 ns	92 a	92 ab	92 ab	92 ns	88 ns
0.065	96	96 a	96 a	92 ab	92	83
0.125	96	96 a	96 a	96 a	97	79
0.25	88	92 a	83 ab	88 ab	88	79
0.5	88	92 a	88 ab	88 ab	88	83
0.75	92	97 a	92 ab	92 ab	92	88
1	83	83 ab	79 ab	83 ab	88	71
1.5	67	63 b	63 b	63 b	63	58
3	83	75 ab	38 c	25 c	83	83
6	80	17 c	0 c	0 d	75	63

Means followed by same letter do not significantly differ (P=.05, Student-Newman-Keuls)

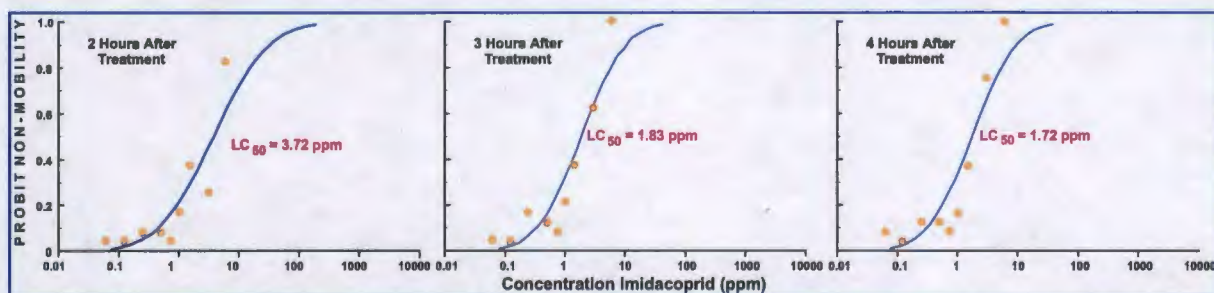


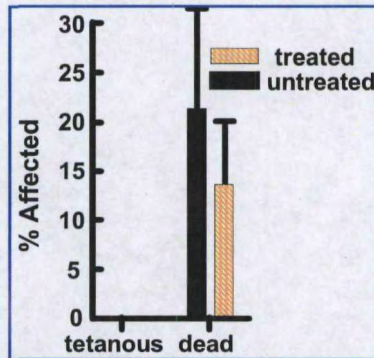
Figure 5 Concentration/response probit non-mobility curves for YOY crabs at 3 post-application intervals after exposure to imidacloprid for 4 hr.

#### (e) 2010 Field trials (Figure 6)

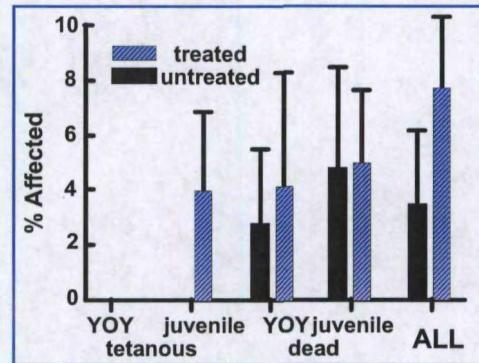
Juvenile crab were collected in Willapa Bay in July the day before each of 2 nearly adjacent 10 ac plots was treated with either Nuprid 2F at 2 lb ai/ac or Mallet 0.5G at 0.5 lb ai/ac. Crabs were confined in small cages (1/2" plastic mesh, 10" diameter, 12" tall, open bottom, gated top) that were buried 3" deep in the sediment. Three or 4 crabs were confined per cage. For the Mallet application, crabs were 1 – 4" carapace width. Two separate size classes were used for the Nuprid application (<1", YOY) and 2 – 4" carapace width. 12 cages placed in each plot and 6 cages within untreated sites ~1000 ft from each plot for each formulation/rate/size class combination (54 cages total). Crab were fresh clams to reduce cannibalism and were monitored for mobility and mortality at 24, 48, and 72 hr after treatment.



Percentages of both dead or tetanous juvenile crab (1 – 4" carapace width), caged and treated in the field with granular imidacloprid (Mallet 0.5G) at 0.5 lb ai/ac were not significantly different from percentages of affected crab similarly caged at a nearby untreated site (Figure 6). Results were similar for two size classes of juvenile crabs treated with liquid imidacloprid (Nuprid 2F) (Figure 7).



**Figure 6** Percentage tetanous or dead caged juvenile crab at 72 hr after field treatment with Mallet 0.5G at 0.5 lb ai/ac.



**Figure 7** Percentage tetanous or dead caged YOY (<1" carapace width) and juvenile (2 – 4" carapace width) crab at 72 hr after field treatment with Nuprid 2F at 2 lb ai/ac.

#### ix) Benthic Infauna

##### (a) Booth unpublished data, 2007

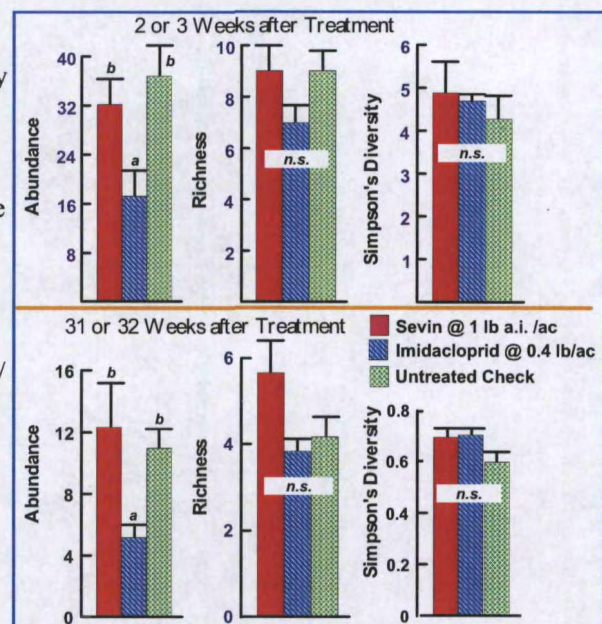
Benthic infauna was sampled in small plot trials treated with imidacloprid (Admire 1.6F; 0.4 lb a.i./ac), carbaryl (Sevin 80SP; 1 lb a.i./ac) or left untreated using 5 cm internal diameter "clam gun" corers to a depth of 15 cm. Six replicate cores were collected per plot, immediately sieved through 0.5 mm mesh, and fixed for 2 – 3 weeks in a buffered formalin solution and then transferred to 75% alcohol. Invertebrates were sorted from debris then identified, mostly to species by Eugene Ruff (annelids), Tricia Towanda (Evergreen University), and molluscs (Pacific Shellfish Institute).

Absolute abundance of non-target invertebrates was significantly lower in plots treated (Figure 8). Neither Species Richness nor Simpson's Diversity differed significantly among treatment plots at both short and long post-treatment intervals.

##### (b) Booth unpublished data, 2008

Benthic infauna was sampled in association with large scale commercial trials in greater detail under Section E (Effectiveness Data) described below. Samples were taken both pre- and post-treatment. Methods were as above, except number of core replicates varied from 6 – 12 among sample sites / dates. Samples were sub-sampled during sorting (50% of each sample was discarded) due to the extreme amount of detritus.

Sixty three taxa were sampled and identified: 32 to species, 10 to genus, 3 to family, 10 to order, 4 to class, 2 to subphylum, and 2 to phylum (Table 14). Imidacloprid did not significantly decrease the absolute abundance, richness (number of taxa), Simpson Diversity, and Shannon Diversity at Bed A90, according to pre- and post-treatment



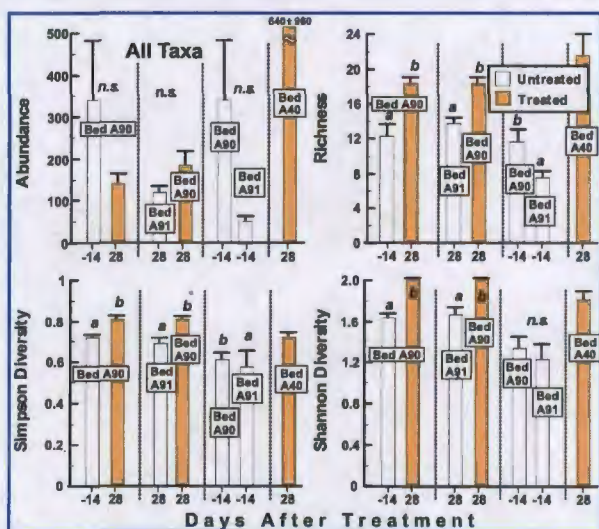
**Figure 8** Affects of carbaryl (Sevin 80S) and imidacloprid (Admire 4.6F) on non-target benthic invertebrates at two post-treatment intervals.

assessments and according to a post treatment assessment between the treated and a nearby untreated bed (Bed A91) (Figure 9). An additional comparison between Bed A90 and the untreated check at 14 days before treatment showed significantly lower richness and Simpson diversity at the latter. Treated Bed A40 is included in the figure. Similar comparisons among the polychaetes (Figure 10), molluscs (Figure 11), and crustaceans (Figure 12) show the same general conclusion: impact of imidacloprid on the benthic infauna was minimal.

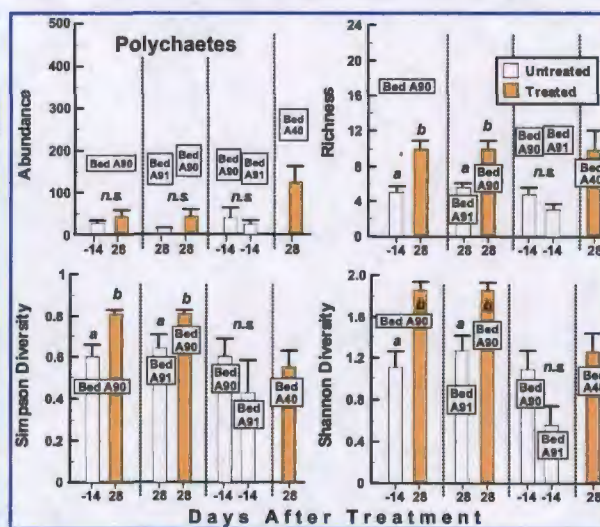
Table 14. List of 63 taxonomic units identified from samples taken from Beds A90, A91, and A40 before and after treatment with imidacloprid in 2008

Phylum Annelida		Order Spionida		Family Cardiidae	
Class Polychaeta		Family Spionidae		Clinocardium sp	41
Family Polynoidae		<i>Polydora cornuta</i>	22	Family Veneridae	
<i>Hesperonoe complanata</i>	01	<i>Pseudopolydora kemp</i>	23	<i>Tapas philippinarum</i>	42
Order Phyllodocida		<i>Pseudopolydora paucibranchiata</i>	24	Family Myidae	
Family Syllidae		<i>Prionospio</i> sp(p)	25	Unidentified Myid	43
<i>Sphaerosyllis californiensis</i>	02	<i>Pygospio elegans</i>	26	<i>Sphenia ovoidea</i>	44
<i>Sphaerosyllis</i> sp(p)	03	<i>Rhynchospio glutaea</i>	27	<i>Cryptomya</i> sp.	44
<i>Typosyllis</i> sp.	04	<i>Streblospio benedicti</i>	28	<i>Mya</i> sp.	45
<i>Exogone dwisula</i>	05	Order Cirratulida		Family Naticidae	
Family Nereididae		Family Cirratulidae		<i>Natica clausa</i>	46
<i>Platynereis bicanaliculata</i>	06	<i>Tharyx parvus</i>	29	Phylum Nemertea	47
<i>Nereis</i> sp(p)	07	Order Opheliida		Phylum Arthropoda – Sub Phylum	
Family Nephtyidae		Family Opheliidae		Crustacea Unidentified crustacean	48
<i>Nephtys caeca</i>	08	<i>Armandia brevis</i>	30	Class Malacostraca	
<i>Nephtys cornuta</i>	09	Order Capitellida		Order Tanaidacea	49
<i>Nephtys cacoides</i>	10	Family Capitellidae		Order Cumacea	50
Family Goniadidae		<i>Barantolla nr americana</i>	31	Order Amphipoda	51
<i>Glycinde picta</i>	11	<i>Capitella capitata</i> hyperspecies	32	Order Mysidacea	52
<i>Glycinde</i> sp(p)	12	<i>Mediomastus californiensis</i>	33	Order Decapoda	
Family Hesioniidae		<i>Notonastus tenuis</i>	34	Unidentified crab megalopoda	53
<i>Podarkeopsis glabrus</i>	13	Family Maldanidae		Class Ostracoda	
Family Phyllodocidae		<i>Sabaco elongatus</i>	35	Order Isopoda	54
<i>Eteone californica</i>	14	Class Oligochaeta	36	Order Ostracoda	55
<i>Eteone spilotus</i>	15	Phylum Mollusca		Class Copepoda	
<i>Eteone</i> sp.	16	Unidentified	37	Order Calanoida	56
<i>Phyllodoce</i> sp(p) [juv]	17	Class Gastropoda		Order Harpacticoida	57
Order Orbiniida		Unidentified (juv)	38	Order Cyclopoida	59
Family Orbiniidae		Class Bivalvia		Class Cirripedia	
<i>Paronella platybranchia</i>	18	Unidentified bivalve	39	Order Thoracica	60
<i>Scolaplos armiger alaskensis</i>	19	Subclass Heterodonta		Barnacle larvae	61
<i>Scolaplos armiger armiger</i>	20	Family Mytilidae		Class Acardia	62
<i>Scolaplos squamata</i>	21	<i>Mytilus trossulus</i>	40	Unidentified Acarida	
				Phylum Hemichordata	
				Class Enteropneusta	63

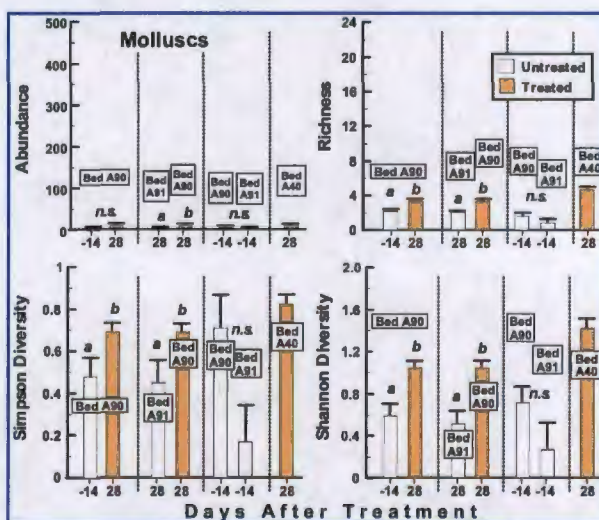




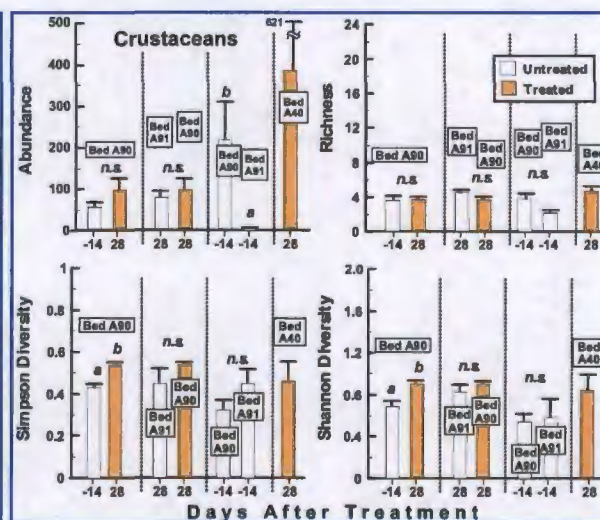
**Figure 10** Impact of imidacloprid treatments on abundance, richness, and two indices of diversity on the 64 benthic invertebrates at Bed A90. Bed A40 is included for comparison.



**Figure 9** Impact of imidacloprid on abundance, richness, and two indices of diversity on polychaetes at Bed A90. Bed A40 included for comparison



**Figure 11** Impact of imidacloprid on abundance, richness, and two indices of diversity molluscs at Bed A90. Bed A40 is included for comparison.



**Figure 12** Impact of imidacloprid on abundance, richness, and two indices of diversity on crustaceans at Bed A90. Bed A40 is included for comparison.

(c) Booth unpublished data, 2010

Benthic infauna was sampled in association with 2 side by side large plot (10 ac) trials of granular and liquid imidacloprid (Mallet 0.5G and Nuprid 2F, respectively) applied at 0.5 and 2.0 lb ai/ac, respectively. Samples were taken both pre- and post-treatment at both plots and at nearby untreated sites. Methods were as above. Results should be forthcoming by February.



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**D) Residue Data****1) Food**

- a) In general: an examination of the USDA PDP (Pesticide Data Program) database for FY2004 and FY2005 showed that only about 25% of food samples had detectable imidacloprid residues. Considering that the acute dietary risk assessment scenario assumed that all imidacloprid commodity residues were at tolerance levels and 100% of all crops were treated, the actual acute dietary exposure would be significantly lower than assessed for the Registration Eligibility Decision (Cutchin 2007).
- b) For fish taken for recreation or subsistence consumption under this proposed EUP and associated program: significant exposures to imidacloprid are unlikely given the limited acreage requested and in light of the rapid dissipation of residues following bed treatment (Felsot and Ruppert 2002).
- c) For shellfish: because most beds will be treated with a planted crop of seed which take multiple years of development prior to harvest, the likelihood of any imidacloprid residues remaining unmetabolized is extremely low, especially in light of its  $K_{ow}$ , as explained in Section F. (Petition for Temporary Tolerance) below.
- d) For oysters: using the fugacity based FISH model and appropriate assumptions, estimates of residues in fish (and hypothetically oysters) ranged on a whole body basis from 0.814  $\mu\text{g/kg}$  to 21.1  $\mu\text{g/kg}$  (the assumed body tissue density was 1  $\text{kg/L}$ ). A detailed explanation for the derivation of these concentrations, as well as exposure estimates, are presented in Section F. (Petition for Temporary Tolerance) below.

**2) Worker Safety**

- a) Exposure estimates for aerial applicators to forest canopy has been calculated at 0.005  $\text{mg/kg/day}$  (Anatra-Cordone, M. and P. Durkin. 2005. Imidacloprid - Human Health and Ecological Risk Assessment – Final Report. Prepared for USDA, Forest Service, Forest Health Protection, GSA Contract No. 10F-0082K, USDA Forest Service BPA: WO-01-3187-0150, USDA Purchase Order No.: 43-1387-4-3131, Task No. 24. Submitted by Syracuse Environmental Research Associates, Inc., 5100 Highbridge St., 42C, Fayetteville, New York 13066-0950)
- b) The re-entry interval (REI) to commercial oyster and clam beds will likely be the same as the labeled REI for other imidacloprid products (e.g., Admire, Guacho) of 12 hours. The 12 hour restriction has limited relevance, as shellfish workers generally have no need to enter sprayed plots for several days, if not weeks, following application. Shellfish beds sprayed at low tides will also be submerged within 12 hours by the intervening high tides, substantially diluting imidacloprid concentrations in water and on substrate.

**E) Effectiveness Data****1) Small plot trials, 2006 – 2008**

Imidacloprid (Admire 1.6F, Bayer Corp.; Imida 2F, Etigra) has been tested for efficacy against burrowing shrimp since 2006 in several small plot (e.g., 3 $\text{m}^2$ , 10 $\text{m}^2$ , 10 $\times$ 20m, or 3 $\times$ 20m) trials, as Washington State EUP acreage limit is 0.1 ac per year. Imidacloprid was sometimes applied along with other with compounds (e.g., flowable sulfur, pyrethrins, and pyrethroids), but was most often compared to carbaryl applied at a lower than standard rate (e.g., 3 vs 8 lb a.i./ac) and an untreated check. In initial (2006) broadcast trials, imidacloprid was effective at a range of rates and at a long post treatment interval (Table 15).

Table 15. Affects of carbaryl (Sevin 80WP), 5 rates of imidacloprid (Admire 1.6F) and an untreated check on # burrows/ $\text{m}^2$  ( $\bar{x} \pm \text{SE}$ ) at 45 and 255 days after treatment (DAT), 2006.

Pesticide	Rate (lb a.i./ac)	Rate	
		45 DAT*	255 DAT
Sevin	3	16.0 $\pm$ 5.5 a,b	17.3 $\pm$ 3.8 a
Admire	0.05	29.7 $\pm$ 9.4 b	38.0 $\pm$ 6.0 b
	1	15.7 $\pm$ 7.1 a,b	18.0 $\pm$ 9.1 a
	2	1.7 $\pm$ 0.9 a	2.0 $\pm$ 1.0 a
	3	1.0 $\pm$ 0 a	0 a
	4	0 b	0
Untreated	0	73.7 $\pm$ 4.9 c	69.7 $\pm$ 6.9 c

\* means followed by the same letter are not significantly different (LSD;  $P=0.05$ ).



Research also include2d the potential of subsurface injection technologies. In 2004 – 2005, we assessed nozzle and spikewheel injection of non-imidacloprid compounds from semi-amphibious vehicles at low tide. In 2006, a 6' wide apparatus holding 4 spikewheels was mounted on a pontoon raft which was pushed over plots with a boat. Imidacloprid was tested multiple times at various rates and locations using the underwater spikewheel technology. Usually, efficacy of imidacloprid was greater (post treatment burrow density was lower) at higher rates, but the response was not always linear. At a test area near Nahcotta, where substrates were primarily sandy, burrow densities were substantially, if not significantly, higher at rates less than 0.2 lb a.i./ac. This was especially true at longer post application intervals (e.g., 42 or 50 days after treatment) (Table 16, Trials 1, 2). Efficacy was not always greater in plots treated with imidacloprid at rates greater than 0.2 lb a.i./ac (Table 16, Trial 2: 2<sup>nd</sup> and 3<sup>rd</sup> post application interval; Trial 5). Burrow density was also significantly lower in plots treated with 2.0 lb a.i./ac carbaryl than in plots treated with 3.0 lb a.i./ac carbaryl (Table 16, Trial 1).

Results of a trial conducted on sandy/silty substrates were confounded somewhat by heavy growths of eel grass (primarily invasive *Zostera japonica*, but also *Z. marina*), which slowed tidal drainage, left standing water on the bed, and obscured burrow counts (Table 17).

Table 16. Affects of carbaryl (Sevin 80SP) and imidacloprid (Admire 4.6F), injected subsurface using underwater spikewheels, on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 5 trials and up to 3 post application intervals (PAI, days after treatment (DAT)) in a sandy substrate at Nahcotta. 2006.

Trial	Treatment	Rate (lb)	Burrow Density*		
			1 <sup>st</sup> PAI†	2 <sup>nd</sup> PAI‡	3 <sup>rd</sup> PAI§
1	Sevin	3	14.7 $\pm$ 3.1 b,c	28.6 $\pm$ 2.9 b	16.4 $\pm$ 1.0 b
	Admire	0.05	23.2 $\pm$ 8.1 c	43.6 $\pm$ 2.9 b	NA
		0.1	5.7 $\pm$ 2.5 a,b	33.1 $\pm$ 2.7 a	NA
		0.2	0.25 $\pm$ 0.2 a	18.2 $\pm$ 1.9 a	13.6 $\pm$ 1.0 a
	Untreated	0	81.0 $\pm$ 2.1 d	91.7 $\pm$ 1.5 c	NA
2	Admire	0.124	23.3 $\pm$ 11.8 a	47.3 $\pm$ 1.6 b	32.4 $\pm$ 1.5 b
		0.25	0.7 $\pm$ 1.2 a	24.9 $\pm$ 3.6 a	17.9 $\pm$ 2.1 a
		0.5	0	22.0 $\pm$ 4.3 a	16.2 $\pm$ 1.9 a
	Untreated	0	62.0 $\pm$ 9.5 b	91.7 $\pm$ 1.5 c	NA
3	Admire	0.2	0.2 $\pm$ 0.2 a	0.7 $\pm$ 0.4 a	NA
	Untreated	0	81.0 $\pm$ 2.1 b	95.3 $\pm$ 3.1 b	NA
4	Admire	0.1	12.2 $\pm$ 2.7 b	NA	NA
		0.2	2.4 $\pm$ 0.7 a	NA	NA
	Untreated	0	72.4 $\pm$ 3.8 c	NA	NA
5	Admire	0.2	6.5 $\pm$ 1.6 a	NA	NA
	Untreated	0	105.4 $\pm$ 4.7 b	NA	NA

\* means followed by the same letter are not significantly different (LSD or t-test; P=0.05).

† Trial 1, 14 DAT; Trial 2, 6 DAT; Trial 3, 10 DAT; Trial 4, 14 DAT.

‡ Trial 1, 42 DAT; Trial 2, 50 DAT; Trial 3, 21 DAT; Trial 4, 21 DAT.

§ Trial 1, 249 DAT; Trial 2, 258 DAT.

Another trial, conducted at the Willapa Bay Fish and Wildlife Refuge, featured applications of imidacloprid (Admire 2F; 0.2 lb a.i./ac) on four different types of substrate. Burrows were counted in four 1 m<sup>2</sup> quadrants within and in a single untreated 1 m<sup>2</sup> plot adjacent to each treatment plot. Shrimp burrow density was significantly lower in all treated compared to untreated plots ( $\bar{x} \pm SE$ ,  $52.2 \pm 15.7$  burrows/m<sup>2</sup>; LSD,  $P=0.05$ ), but was significantly higher in a plot of silty hummocks than in plots of other substrate types (Table 18). In 2007, three broadcast trials continued to demonstrate the fast action and fairly long-lasting efficacy of imidacloprid on burrow density (Table 19).

Table 18. Affects of imidacloprid (Admire 1.6F) at 0.2 lb ai/ac on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) on different substrate types at 13 days after treatment, 2006.

Treatment	Substrate	Burrow Density*
Admire	Oyster Shell	$2.8 \pm 0.6 a$
	Silt	$3.2 \pm 3.2 a$
	Sand / Silt	$8.8 \pm 4.3 a$
	Silt Hummocks	$19.0 \pm 0.6 a$

\* means followed by the same letter are not significantly different (LSD;  $P=0.05$ ).  
Untreated check ( $52.2 \pm 15.7$ ) not included in analysis

Table 17. Affects of imidacloprid (Admire 1.6F) on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) at 10 days after treatment in sand / silt at Middle Island Sands, 2006.

Treatment	Rate (lb a.i./ac)	Burrow Density*
Admire	0.2	$4.2 \pm 2.0 a$
	0.4	$8.1 \pm 1.7 a$
Untreated	0	$33.5 \pm 2.6 b$

\* means followed by the same letter are not significantly different (LSD;  $P=0.05$ ).

Table 19. Affects of imidacloprid (Imida 2.F) on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 3 trials and at 2 post application intervals (PAI, days after treatment (DAT)) at Nahcotta, 2007.

Trial	Treatment	Rate (lb a.i./ac)	Burrow Density*	
			1 <sup>st</sup> PAI †	2 <sup>nd</sup> PAI ‡
1	Imida	0.5	0	0
		0.25	$0.2 \pm 0.1 a$	$1.8 \pm 0.9 b$
		0.125	$2.9 \pm 1.1 a$	$18.3 \pm 4.5 b$
	Untreated	0	$119.5 \pm 2.4$	$71.7 \pm 2.4 c$
2	Imida	0.5	0	$1.3 \pm 0.7 a$
		0.25	$6.3 \pm 3.1 b$	$15.0 \pm 4.7$
	Untreated	0	$26.1 \pm 4.8$	$71.7 \pm 2.4$
3	Imida	0.5	$7.5 \pm 1.6 a$	$5.8 \pm 2.5 a$
		0.25	$16.2 \pm 2.3$	$48.9 \pm 6.2 b$
	Untreated	0	$85.6 \pm 3.9$	$94.7 \pm 5.2 c$

\* means followed by the same letter are not significantly different (LSD;  $P=0.05$ ).

† Trial 1, 7 DAT; Trial 2, 25 DAT; Trial 3, 2 DAT

‡ Trial 1, 99 DAT; Trial 2, 45 DAT; Trial 3, 12 DAT

Other small plot trials conducted in 2007 and 2008 examined the efficacy of imidacloprid when spikewheel injected by boat or ATV, sediment type, and eelgrass cover on the efficacy of imidacloprid (Table 20). None of the sites featuring application by spikewheel showed outstanding control, whereas burrow density was reduced by  $\geq 95\%$  compared to burrow density in untreated plots when application was by broadcast.

Table 20. Affects of sediment type, application timing, and application method on efficacy of imidacloprid (0.5 lb a.i./ac) against borrowing shrimp (% reduction in burrows in treated compared to untreated plots). Each row represents a separate experiment.

Sediment Type / Timing	Burrow Density in Untreated Plots (#/m <sup>2</sup> )	Percentage burrow reduction		
		Spikewheel on ATV	Spikewheel on Boat	Broadcast
Sand / April	24	16		62
Sand / May	24	72		62
Sand / July	24	83		96
Sand / September	24	25		95
Silt / June	79		0	49
Sand / June	18		0	96
Eelgrass on sand / August	11	48	74	37
Eelgrass on sand / August	28		0	9



Other trials that featured application by spikewheel lacked a comparison with a broadcast application were conducted on beds with a thin eelgrass cover (Table 21). These trials demonstrated moderate to poor reduction in burrow density, with generally lower efficacies when applications were in August.

2) Large scale commercial trials, 2008

a) Methods

(1) Applications

Applications were made according to a Federal Use Permit and accompanying experimental label approved by the EPA. Both contained Directions for Use and Restrictions that were similar to those in the 24C label for use of the standard material, Sevin™, on oyster beds (i.e., do not harvest clams or oysters within one year after treatment, proper and visible flagging of beds, a 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray, during aerial applications, all public access areas within one-quarter (¼) mile and all public boat launches within a one-and-a-half (1½) mile radius of any bed scheduled for treatment shall be posted). The experimental treatments were applied as similarly as possible to those made for the conventional carbaryl-based program and required the collaboration of the commercial applicator, Dan Foster, and the director of the carbaryl program, Dennis Tufts.

Experimental beds were proposed by grower collaborators and selected based on degree of shrimp infestation, size, and proximity to untreated areas or beds treated with Sevin. A 20 ac bed located near the mouth of the North River (A90) had been fallow for at 12 years, had a moderate to heavy shrimp infestation and was isolated from other shellfish beds, so provided a good site to study both efficacy and non-target impact to salmonids. A 10 ac bed near the mouth of the Cedar River (A40) was also used as a site to assess both non-target impact and efficacy. A105 was located in between these sites and had the additional advantage of being accessible from shore. Two smaller beds were located in the Stoney Point growing area (B242 and B183). Two beds were also located in the Oysterville and Nahcotta growing areas (E148 and E163, respectively) where substrate is sandier than the primarily silty substrate of the northern and eastern areas of Willapa Bay. The original intent to match all beds with a nearby untreated area could not always be met. All beds except A105 were inspected prior to application for burrow density, dominant substrate type, amount and kind of eelgrass cover, and other attributes (Table 22).

Table 21. Affects of application timing on efficacy of imidacloprid applied using spikewheels on ATV(0.5 lb a.i./ac) against burrowing shrimp (% reduction of burrows in treated compared to untreated plots). Each row represents a separate experiment.

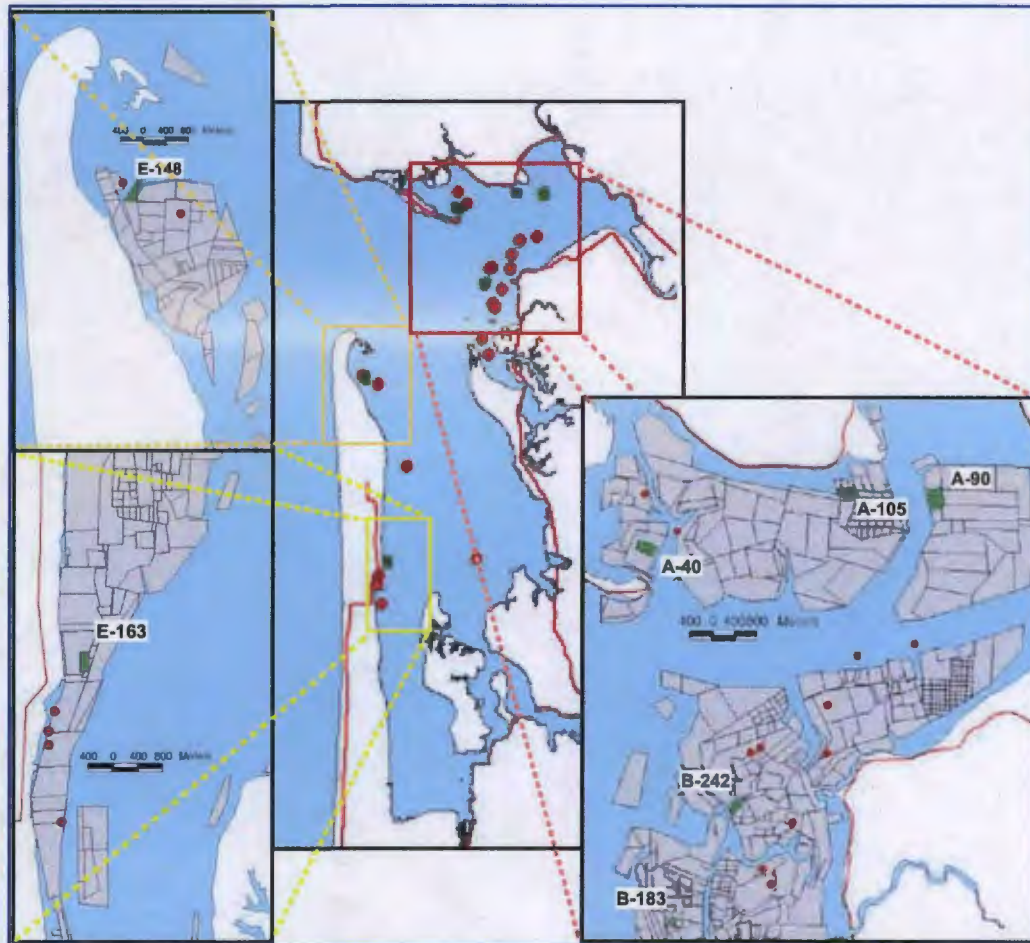
Burrow Density in Untreated Plots (#/m <sup>2</sup> )	Percentage burrow reduction	
	July	August
12	83	
13	87	
29		17
11		72
28		0

Table 22. Attributes of commercial oyster beds treated with imidacloprid in Willapa Bay, 2008.

COMPANY	BED NAME	SIZE (ac)	STAGE <sup>a</sup>	LAST TRT <sup>b</sup>	PLANT DATE <sup>c</sup>	ELEV <sup>d</sup>	SP <sup>e</sup>	CUL T <sup>f</sup>	SUB <sup>g</sup>	EEL GRASS <sup>h</sup>	LAT <sup>i</sup>	LONG <sup>j</sup>
Nisbet Oyster	A40	10.0	Cedar R	2006	2008	0.3	G/M	S/H	S/I	heavy †	46.71417	-123.95542
Coast Seafood	A105	10.0	Cedar R	2002	2008	-0.5	M/G	S/H	M/I	heavy †	46.72493	-123.93408
Taylor Shellfish	A90	20.0	Cedar R	pre-95	none	-0.5	G/M	S/H	S/I	patchy †	46.43240	-123.53940
Nisbet Oyster	B242	6.0	Cedar R	2005	2007	1.0	G	S/H	S/I	none †	46.67035	-123.94487
Nisbet Oyster	B183	4.0	Cedar R	2005	2008	1.5	G/M	S/H	M/I	patchy †	46.65178	-123.95228
Northern	E148	10.0	Sheldon	½-'03, ½-none	2008	1.0	G/M	S	G/M/S	50% ‡	46.61520	-124.04040
Taylor Shellfish	E163	10.0	Sheldon	never	2008	0.5	G	S/H	S	patchy ±	46.51505	-124.01963

<sup>a</sup> Helicopter staging area, <sup>b</sup> Year last treated, <sup>c</sup> Year and type of planting, <sup>d</sup> Bed elevation, <sup>e</sup> Species of shrimp (G-ghost, M-mud, G M-ghost dominant, M G-mud dominant), <sup>f</sup> Cultural Type (S-seed, H-harvest, LL-long line, <sup>g</sup> Substrate (M-Mud, S-Sand, I-Silt, G-gravel), <sup>h</sup> approximate density of either (†)native (*Zostera marina*) or ‡ Japanese (*Z. japonica*) density, <sup>i</sup> Latitude (decimal degrees), <sup>j</sup> Longitude (decimal degrees)

Imidacloprid was applied aerially using helicopters to 7 commercial shellfish beds on July 2, 2008 in conjunction with applications of the Sevin, which was applied on July 2, 3, or 7 depending on bed location (Figure 13).



**Figure 13.** Name, location, size and shape of commercial oyster beds treated with imidacloprid (green) relative to locations of beds treated with carbaryl (red circles indicate points of entry).

Imidacloprid was applied at a rate of 0.5 lb a.i. per ac to 5 of the 7 beds. Due to a mistake, beds in the Oysterville and Nahcotta growing areas were treated at 0.25 lb a.i. per ac. To test the affects of a second half-rate treatment, one half of Bed E163 was treated again 5 days later on July 7. Two types of ground applications were also tested on the E163 bed: 1) subsurface injection using five Spikewheels™ pulled behind an Argo™ Track ATV and 2) application using 27' spray boom, also mounted on the Argo. Plot sizes were 2 and 5 ac, respectively. Application rate was 0.5 lb a.i. per ac on 1 August.

#### (2) Observations of burrowing shrimp

At all but one site, shrimp burrows were counted both before and at 4 weeks after treatment within a square meter grid placed along transects that criss-crossed the bed diagonally at distance intervals of 5, 10, or 15 paces depending on plot size, to give samples of 30 or more counts per bed. High flood tides sometimes constrained sample size. Counts were averaged within each half transect for statistical analysis.



### (3) Observations of impact to non-target macrofauna

Number of live, dead, or otherwise impaired but visible macrofauna were counted along transects at 5 shellfish beds following the applications. The area at each observation point was roughly 4 m<sup>2</sup> (2 m<sup>2</sup> to the front right and left plus 2 m<sup>2</sup> to the rear right and left). The entire bed could not be covered due to time limitations, but the transects usually crossed the beds diagonally so observations were made at both low and high ends and at both sides. The number of paces between observation points, and consequent total number of observations, varied according to bed size and duration of the low tide. Three beds, two treated with imidacloprid and one treated with carbaryl, were examined within 1 hr after application. An untreated area near one of the imidacloprid-treated beds that was of similar bed elevation, substrate type, and vegetation cover was also examined as a check. Five beds (2 treated with imidacloprid, 2 treated with carbaryl, and the same untreated bed neighboring the imidacloprid-treated bed) were examined at 24 hrs after treatment.

### (4) Water samples

Water was sampled for analysis of imidacloprid concentration directly on the bed of three beds and in the adjacent channels of two beds. On-bed samples were taken by grab near the center of the bed, initially when depth of the in-coming tide reached 6" and on subsequent high tides at mid-depth of the water column. In-channel grab samples were taken at both maximum low and high tides at mid-depth of the water column. All samples were held on ice and extracted for imidacloprid analysis within 7 days by Pacific Agricultural Laboratories, Portland, OR.

## b) Results

### (1) Burrowing shrimp

Burrow density varied substantially at all aerially treated beds, both before and after treatment with imidacloprid (Figure 14). In general, burrow density was significantly lower in beds after treatment with imidacloprid, but levels were not low enough to allow oysters to survive. At the A90 site, burrow density declined significantly from 13.9 at 14 days before treatment to 8.1 at 29 days after treatment (DAT) but was high again 30 days later at 59 DAT. Burrow density also declined in the first 29 DAT, although not significantly, in the nearby untreated area. Due to its drainage patterns and proximity to the North River and a major channel, A90 had a much less regular surface than most other shellfish beds. Burrows on the myriad of small hummocks had been exposed for longer and were much more visible than burrows under water. At 58 DAT, mean burrow density on exposed ground was 12.1 compared to 8.9 on ground under ½ or more inches of water. At A40, number of burrows per m<sup>2</sup> apparently declined to an acceptable level (4.4 burrows/m<sup>2</sup> at 29 DAT and 3.1 burrows/m<sup>2</sup> at 58 DAT), but heavy covers of native eelgrass and algae complicated assessments and could have caused some burrows to be missed. The lack of an adequate untreated control site near A40 also confounded interpretation of results. A similar scenario occurred at B242: burrow density apparently declined significantly and to a potentially acceptable level after treatment with imidacloprid, but heavy vegetation and the lack of a nearby untreated area for comparison confounded the experiment. At B183, burrow density declined in the bed treated with imidacloprid, but also declined in a nearby untreated area in the first 29 DAT. However, the check at B183 was close enough to the treated area that it could have been contaminated by off-site drift. Bed E148, treated with the half rate of imidacloprid, initially showed a similar scenario as that at the A90 site: burrow density was significantly lower at 30 DAT compared to 10 days before treatment, but was still not at an acceptable level for planting. Burrow density was measured as lower at 63 DAT, but not all sections of the bed were examined. A more thorough examination of the bed at 104 DAT gave a higher burrow density.

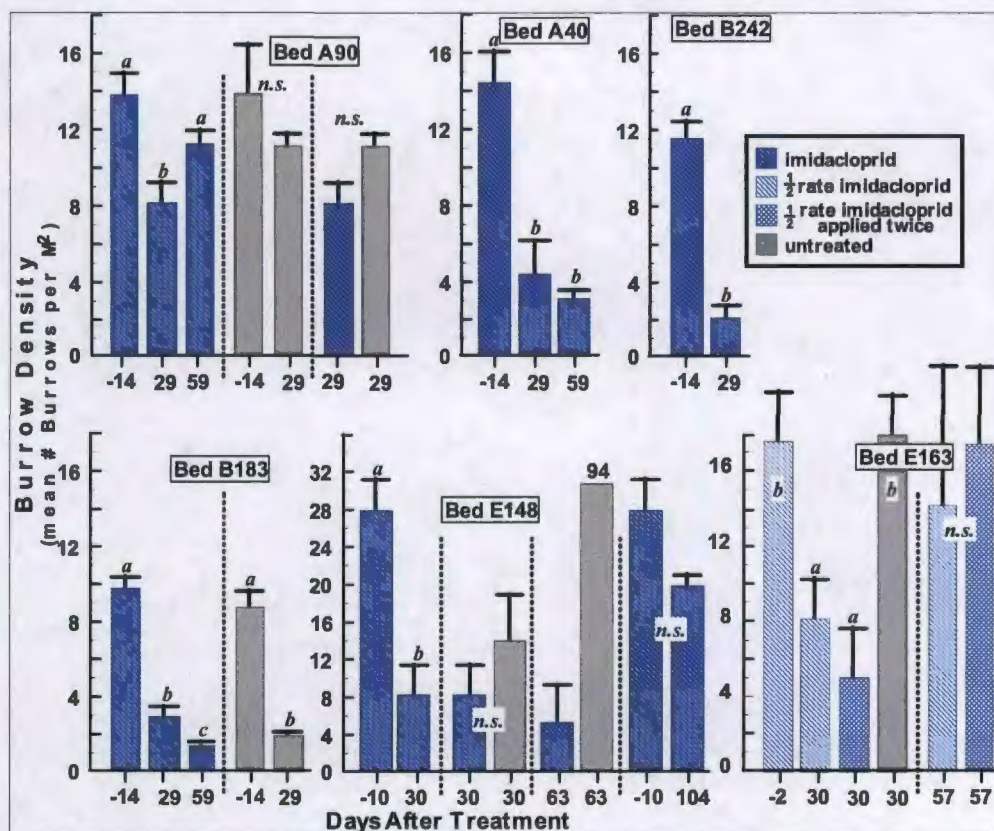
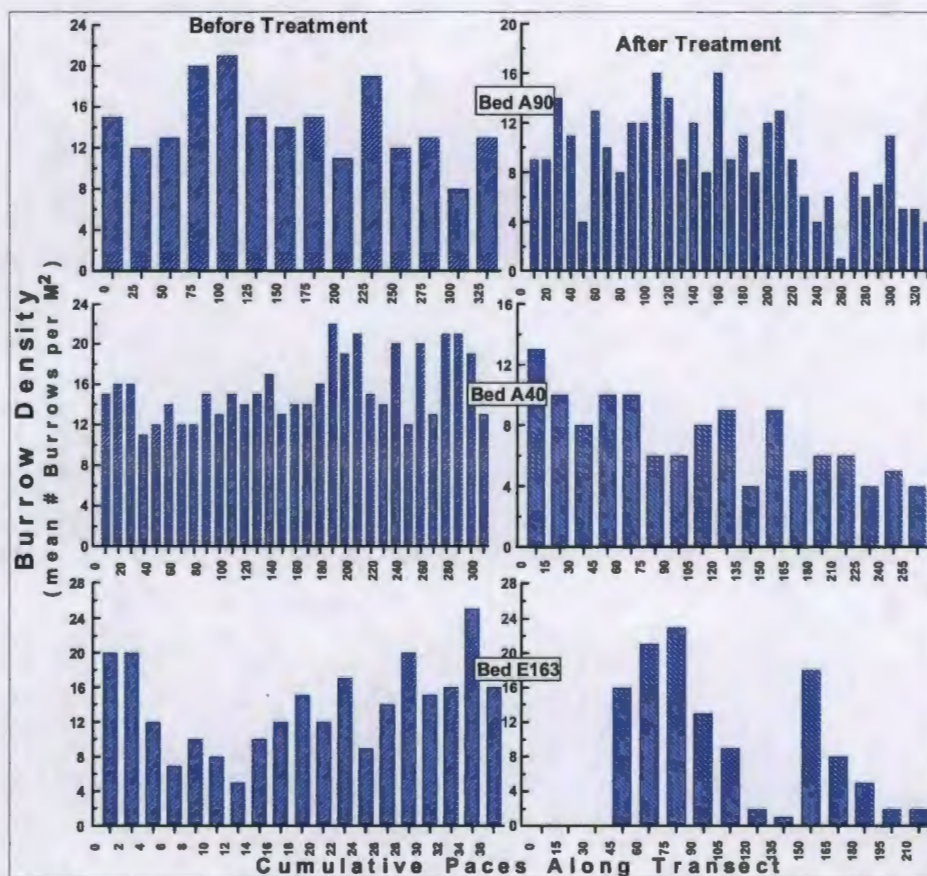


Figure 14. Effects of imidacloprid on burrowing shrimp density at 2, 10, or 14 days before and at 4, 8, or 10 weeks after treatment. Letters "a" and "b" indicate significantly different densities (n.s., not significant).

Shrimp burrow density was also quite variable within beds, especially post-treatment. Some portions of the bed showed moderate burrow density, but other sections were nearly barren. At the first post treatment assessment, comparisons of burrow densities along the transects at some beds showed relatively highly variable post-treatment distributions of shrimp burrows, especially at E163 (Figure 15). At Bed A148, four strips of relatively low burrow density (9.2, 8.3, 1.8, 1.7 per m<sup>2</sup> at a third post-treatment assessment (58 DAT)) were interspersed among stretches of higher burrow density (not counted). Burrow densities at a nearby untreated site were significantly higher (94.4 per m<sup>2</sup>).





**Figure 15** Variation in burrow density along sampling transects at the beds treated with imidacloprid.

Additional observations at E163 showed the patchy distribution of burrow counts to be associated with vegetation, substrate elevation, and related patterns of tidal drainage (Figure 16). At Bed A148, four strips of relatively low burrow density (9.2, 8.3, 1.8, 1.7 per  $m^2$  at a third post-treatment assessment (63 DAT)) were interspersed among stretches of higher burrow density (not counted). The width of these strips (~18 ft) is similar to the width of a spray strip. Burrow densities at a nearby untreated site were significantly higher (94.4 per  $m^2$ ).



**Figure 16** Distribution of burrow counts among bed attributes at E163 and 63 DAT.

The ground applications at E163 showed significant reductions in burrow densities in plots treated using either Spikewheels or spray boom compared to both pretreatment levels and densities in an adjacent untreated plot (Figure 17).

(2) Impact to non-target macrofauna, primarily crab  
No visibly affected fish were observed. Although a few dead nereid polychaetes were observed at the A90 and the E163 beds, crabs (Dungeness, rock, and hermit) were observed as the primary animal impacted by imidacloprid (Table 23). Affected crabs were not dead, but in a state of tetanus shock. They were either entirely exposed or only partially buried and moved very sluggishly when disturbed. Legs and mouthparts were extended and trembled. In comparison, more crab were affected on beds treated with carbaryl and all were dead. Almost all crab were observed in lower areas of the bed or off-bed.

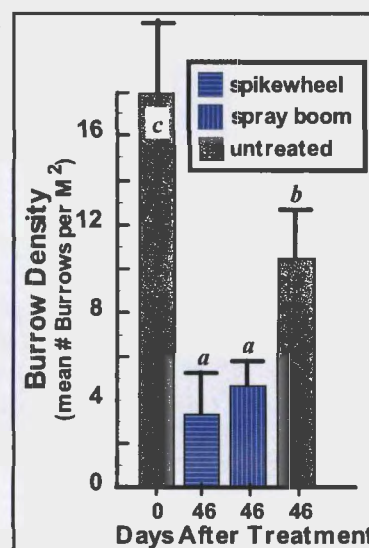


Figure 17 Burrow density in large plots treated with imidacloprid using spikewheels or spray boom.

Table 23. Impact of imidacloprid (imid), carbaryl, or no treatment (untreated) on crab, as observed visually at 1 or 24 hours after treatment (HAT).

Bed	Treatment	Treatment Date	HAT	Transects	Paces Between Observations	Observations	Number Crab		
							Normal	Tetanus	Dead
A90	imid	July 2	1	3	1	500	0	0	0
A91	untreated		1	3	1	683	0	0	0
A40	imid	July 2	1	4	5	146	0	0	0
E147	carbaryl	July 7	1	5	1	500	0	0	3
A90	imid	July 2	24	6	5	204	0	15	0
A91	untreated		24	2	5	46	0	0	0
A40	imid	July 2	24	4	5	79	0	6*	0
B183	imid	July 2	24	2	5	65	2	3**	0
E163	imid	July 2	24	7	1	700	0	1	0
A100	carbaryl	July 7	24	4	10	69	3	0	100***
A79	carbaryl	July 7	24	3	20	60	0	0	25****

\* also 10 – 15 lethargic and attenuating crab submerged in drainage channel off lower end of bed.

\*\* also 4 – 8 lethargic and attenuating crab submerged in drainage channel off bed.

\*\*\* also ~ 100 dead crab in 3x5 m section of drainage channel off lower end of bed.

\*\*\*\* rapidly rising tide prevented off-bed observations.

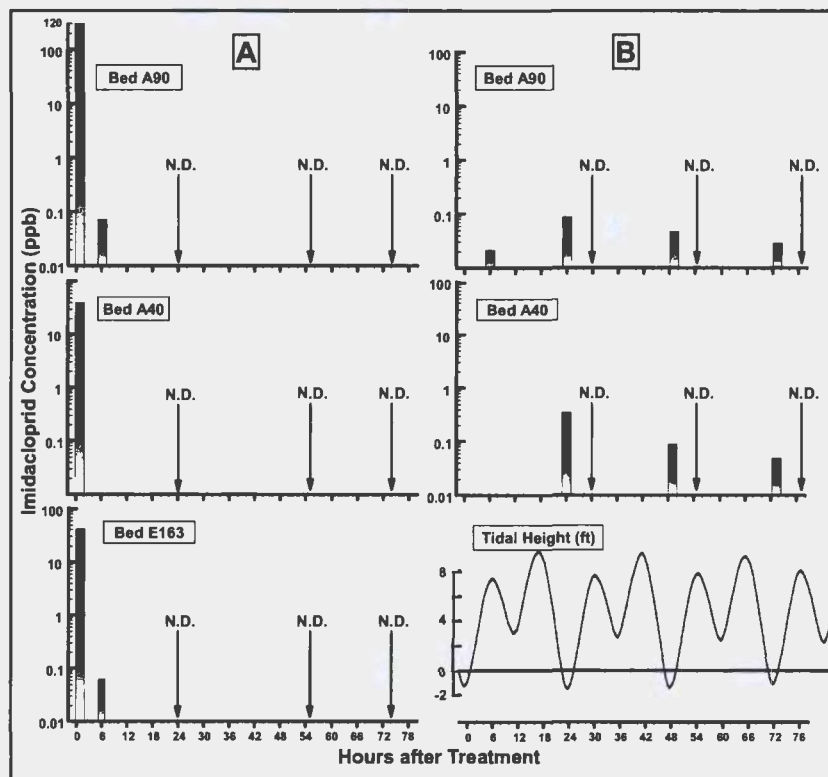
### (3) Impact to non-target benthic infauna

Results are described above in Section C (Toxicity Data).

### (4) Water Samples

Concentrations of imidacloprid sampled over the beds dropped precipitously between 1 and 6 hours after treatment (HAT) and were not detected afterward (Figure 18). Concentrations in the channels adjacent to the beds were recovered from both sample sites at 6 and 24 HAT and at 49 and 74 HAT at one of the sites. These timings were synchronized to the high tides.





**Figure 18.** Concentrations of imidacloprid in water sampled (A immediately over the bed, B) in adjacent channels after applications at ~6 a.m. July 2 and C) tidal fluctuations during the same time at Toke Point near Beds A90 and A40. N.D., not detected (Method Reporting Limit = 0.02 ppb).

### c) Discussion

The general failure of the aerial applications of imidacloprid to suppress burrowing shrimp densities to commercially acceptable levels was due to several factors. The water samples indicated that at least some imidacloprid was transported off-bed during high tide, which likely contributed to generally poor on-bed efficacy against burrowing shrimp relative to carbaryl. Imidacloprid has a lower coefficient of adsorption than carbaryl, so does not bind as tightly to sediments, especially silt, a major component of Willapa Bay tidelands. In addition, most of the beds where efficacy was poor were blanketed with thick vegetation which likely inhibited penetration of imidacloprid. Percent cover of native eelgrass (*Zostera marina*) averaged 67% on Bed A40 and 47% on Bed B183 during pre-treatment assessment while average percent cover of Japanese eelgrass (*Z. japonica*) was 37% on E163. Cover of eelgrass and sea lettuce (*Ulva sp.*) increased during late summer and frequently exceeded 100% in many of the m<sup>2</sup> grids, which greatly confounded measurement of shrimp burrows. At A90, the currents from the North River may have contributed to the already strong tidal currents to wash imidacloprid from the bed before kill. Rising tides approach B183 from both east and west so imidacloprid may not have been washed away as quickly there, resulting in relatively better efficacy. B183 had also been recently dredged so may have retained imidacloprid longer. Impact to non-target macro-fauna was mostly limited to crab and apparently to a smaller portion of the on-bed population compared to carbaryl.

## 3) Small plot trials, 2009

Several studies of the potential of imidacloprid to suppress burrowing shrimp were conducted using small plots (10×10m, 30×30m) in accordance with a Washington State Experimental Use Permit. Objectives were to compare effects of formulation, surfactants, eelgrass cover, substrate, and season.

Early season trials focused on the affects of higher rates and different formulations than the 2F formulation (Nuprid™, NuFarm Inc) at 0.5 lb a.i./ac. Results indicated that 5% and 1% granular formulations of imidacloprid (Mallet 0.5G™ and Mantra 1G™, respectively, both NuFarm Inc.) were highly effective, both alone and when combined with reduced rates of carbaryl (Sevin 80S™, Bayer Corp.) (Tables 24, 25).

Table 24. Affects of imidacloprid formulated as a 5% or 1% granular (Mallet 0.5G, Mantra 1G, respectively) or 2 lb/gal flowable (Nuprid 2F) applied alone or in combination with an 80% wettable powder formulation of carbaryl (Sevin 80WP) on burrowing shrimp (# burrow / m<sup>2</sup>), Spring 2009.

Treatment	Rate (lb a.i./ac)	Burrow Density*	
		Pre-treatment †	Post-treatment ‡
Mallet 0.5G	2.0	44.4 <i>n.s.</i>	0.2 <i>a</i>
Mallet 0.5G	1.0	53.2	1.2 <i>a</i>
Mallet 0.5G	0.5	49.6	0.3 <i>a</i>
Nuprid 2F	0.5	56.0	1.2 <i>a</i>
Nuprid 2F	1.0	57.2	0.5 <i>a</i>
Nuprid 2F	2.0	50.8	0 <i>a</i>
Nuprid 2F+Sevin 80WP	0.5 / 2.0	51.2	2.3 <i>a</i>
Nuprid 2F+Sevin 80WP	0.5 / 4.0	50.8	0.3 <i>a</i>
Mantra 1G	1.0	360	0.2 <i>a</i>
Untreated	0	49.2	38.7 <i>b</i>

\* means followed by the same letter are not significantly different (LSD; P=0.05).  
† 4 days before treatment, 4/23/09  
‡ 8 days post treatment, 5/6/09

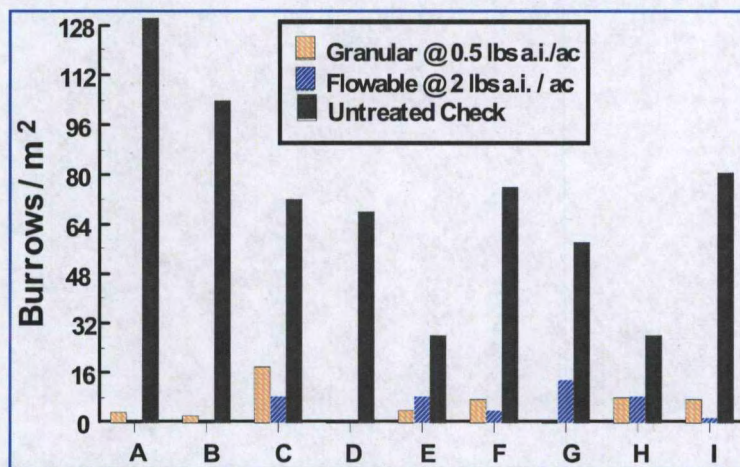
Table 25. Affects of formulation and rate of imidacloprid on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 3 trials and at 10 – 12 days after treatment at Ellen Sands (Trial 1), Sherwood (Trial 2), and WDFW (Trials 3,4), Spring 2009.

Trial	Treatment	Rate (lb a.i./ac)	Burrow Denisty*	Comments
1	Nuprid 2F	2.0	3.2 ± 0.8 <i>a</i>	sandy, silt substrate
	Mallet 0.5G	0.50	20.4 ± 3.2 <i>a</i>	
	Untreated	0	96.4 ± 2.8 <i>b</i>	
2	Nuprid 2F	2.0	7.6 ± 0.8 <i>a</i>	sandy, silt substrate light eelgrass
	Mallet 0.5G	0.50	4.0 ± 1.2 <i>a</i>	
	Untreated	0	31.2 ± 1.2 <i>b</i>	
3	Nuprid 2F	2.0	26.4 ± 2.4 <i>a</i>	sandy, silt substrate tidal flow
	Mallet 5G	0.50	27.6 ± 2.0 <i>a</i>	
	Untreated	0	78.8 ± 2.4 <i>b</i>	
4	Nuprid 2F	2.0	5.2 ± 1.2 <i>a,b</i>	sandy, silt substrate thick eelgrass cover
	Mallet 0.5G	0.50	14.6 ± 2.0 <i>a</i>	
	Untreated	0	30.8 ± 1.6 <i>b</i>	
5	Nuprid 2F	2.0	14.2 ± 1.2 <i>a</i>	sandy, silt substrate thick eelgrass cover
	Mallet 0.5G	0.50	27.6 ± 2.0 <i>a</i>	
	Untreated	0	30.8 ± 1.6 <i>b</i>	

\* means followed by the same letter are not significantly different (LSD; P=0.05).



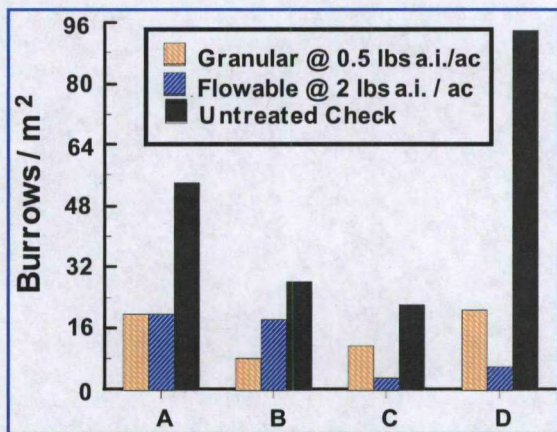
In 9 small plot trials conducted throughout the season, both the granular and flowable formulations of imidacloprid suppressed densities of shrimp burrows compared to an untreated check, but neither formulation was consistently more effective than the other (Figure 19).



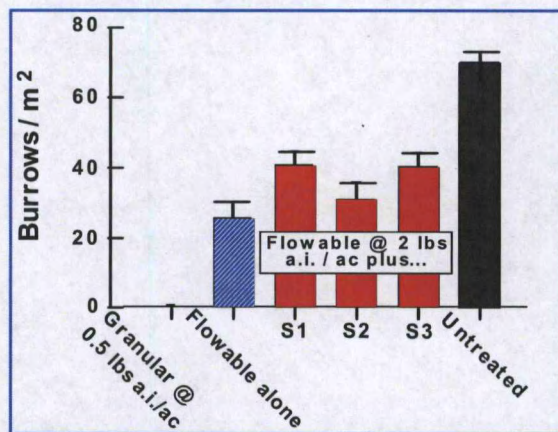
**Figure 19** Effects of a 0.5% granular and 2F flowable formulation of imidacloprid on burrowing shrimp in 9 studies.

Levels of suppression were similarly inconsistent among four trials conducted on beds heavily infested with Japanese eelgrass (Figure 20).

Another trial addressed the potential of 3 surfactants to improve efficacy of the flowable imidacloprid. Burrow density was significantly lower in plots treated with the granular material at a rate of 0.5 lb a.i. per ac than in plots treated with the flowable at 2 lbs a.i. per ac, even when surfactants were added to the latter (Figure 21).



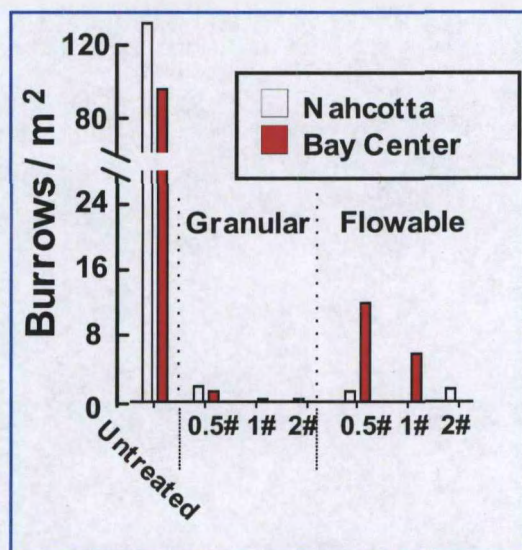
**Figure 20** Effects of 0.5% granular and 2F flowable imidacloprid on burrowing shrimp in 4 studies conducted on ground infested with Japanese eelgrass.



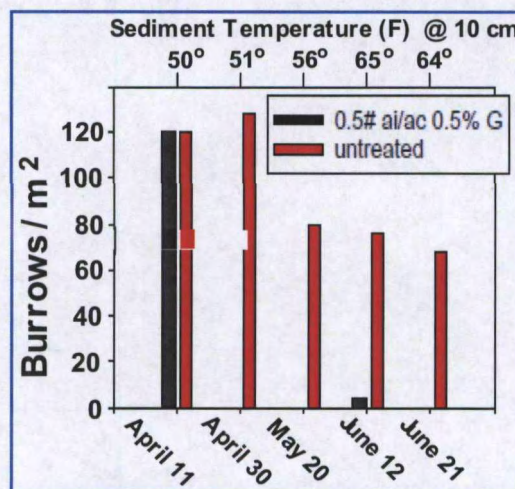
**Figure 21** Effects of three surfactants on flowable imidacloprid compared to granular imidacloprid and an untreated check.

Efficacy was also compared between the Nahcotta and Bay Center areas. Burrow density was substantially reduced compared to untreated sites in both areas, especially by the granular formulation (Figure 22). The flowable, however, was more effective in Nahcotta.

Aside from one early-season trial, granular imidacloprid applied at 0.5 lbs a.i. per ac significantly reduced densities of shrimp burrows compared to an untreated check, regardless of application date (Figure 23).



**Figure 22** Effects of two study site location on the efficacy of granular and flowable formulations of imidacloprid applied at three rates.



**Figure 23** Effects of season on density of shrimp burrows treated with granular imidacloprid compared to an untreated check.

#### 4) Large scale trial, 2009

In response to poor efficacy demonstrated in the 2008 large scale trials of flowable imidacloprid applied at 0.5 lb a.i. per ac, plans for the 2009 large scale trials included application of the flowable formulation at a rate of 2.0 lb a.i./ac. An application for Federal Experimental Use Permit was submitted to the EPA in May 2009, but the Environmental Fates Division requested more time to review the application. In the meantime, a granular formulation of imidacloprid had demonstrated good efficacy in small plot (0.02 ac) trials. Discussions with the EPA lead to an exemption from a FEUP that allowed application of the granular formulation to 10 ac at a rate of 0.5 lb a.i./ac. The exemption ultimately lead to applications to three plots at two sites: 1) a 9 ac plot in the Cedar River area, 2) a ½ ac plot placed near to the 9 ac plot, and 3) a ½ ac plot at a site off the Bay Center Peninsula. Small plot trials were allowed under a Washington State Experimental Use Permit and were included at both A43 and B313 to compare granular and liquid formulations of imidacloprid.

##### a) Objectives

- Assess the efficacy of granular imidacloprid against burrowing shrimp at a commercial scale plot
- Compare the efficacies of granular imidacloprid at a different site with differing substrate
- Compare efficacies of liquid imidacloprid in smaller plots
- Assess the impact of imidacloprid at the commercial scale on non-target fish<sup>1</sup>
- Measure the associated concentrations of imidacloprid in the water column and in sediments
- Measure sediment grain size and total organic carbon as another factor that could effect efficacy

<sup>1</sup> Studies of non-target impact to fishes are described elsewhere



## b) Methods

## (1) Study Sites

A 9 ac plot located near the Cedar River Channel in North Willapa Bay (Figure 24) was treated with granular imidacloprid at 0.5 lb a.i. per ac on July 21. Two additional ½ ac plots, one near the 9 ac plot on Bed A43 and another on Bed B313 north of Bay Center, were treated with granular imidacloprid at 0.5 lb a.i. per ac. Two more smaller plots (<0.1 ac) at each location were treated with flowable imidacloprid at 2.0 lb a.i./ac.

The substrate on about 60% of the southern portion of the 9 ac bed was barren of vegetation whereas the northern end was densely covered by the native eelgrass, *Zostera marina*. During late July, the eelgrass trapped filamentous algae, which continued to grow into mid-July but began to die in mid-August. Both the eelgrass and algal mats confounded assessments of shrimp burrows somewhat (i.e., lowered counts by 10 to 20%). Both ½ ac plot on Bed A43 the ½ plot of Bed B313 were barren of vegetation. The shrimp community on Bed A43 was comprised of ghost shrimp (*Neotrypaea californiensis*), the California ghost shrimp (*N. gigas*), and likely mud shrimp (*Upogebia pugettensi*). Cockles were also present. The macro invertebrate community at Bed B313 was comprised entirely of ghost shrimp.

## (2) Efficacy

At the 9 ac bed, burrows were counted inside m<sup>2</sup> grids placed along transects that crossed the large bed at 2 pre-treatment and 2 post-treatment intervals. At the ½ ac plots, burrows were counted within a m<sup>2</sup> grid placed within each of 16 4 m<sup>2</sup> grids within the plot. Additional counts were made at the 9 ac bed at 30 days after treatment inside 1 ft<sup>2</sup> rings, which were then excavated using clam guns. Seven double clam gun cores (2 cores at the same spot) were taken within each ring and macro invertebrates were identified and counted. Three rings were sampled within each of the areas with and without eelgrass.

## (3) Imidacloprid concentrations in water

As in 2008, water was sampled both on-bed and in adjacent channels at ~2 hr after treatment (depth of incoming tide was 6") and on the low and high tides for 3 days after treatment. Duplicate samples were taken at some sites and times. In 2009, water was sampled at 5 on-bed locations.

## (4) Imidacloprid concentrations in sediments

Sediments were sampled and analyzed for imidacloprid at 1) a single site within the bed immediately before treatment, 2) a single site within the bed and at three areas outside the bed at 1 day after treatment, and 3) at 6 within-bed sites (3 in the southern half where no vegetation was present and 3 in the northern end under thick blankets of eelgrass) and 5 sites outside the treated area. Sediments were sampled to a depth of 10 cm using 5.1 cm internal diameter PVC corer. Three cores were combined and homogenized to comprise a single replicate sample.

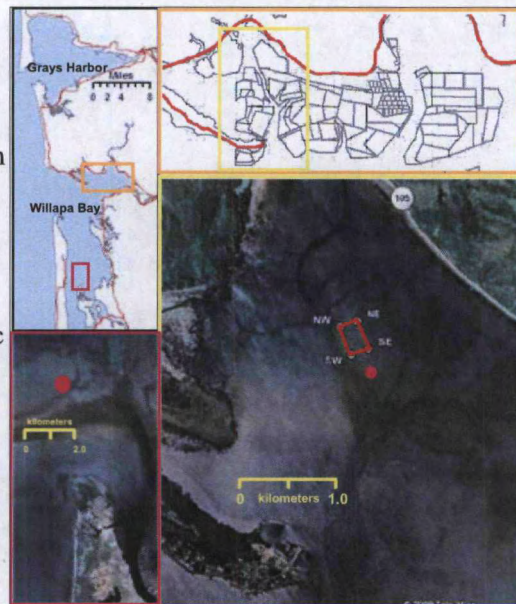
## (5) Sediment grain size and total organic carbon (TOC)

Sediments for grain size and TOC analyses were sampled according to the same protocols as for imidacloprid analysis. Grain size was measured at the WSU Longbeach Research Unit. TOC was measured at Analytical Resources Inc., Tacoma, WA..

## c) Results

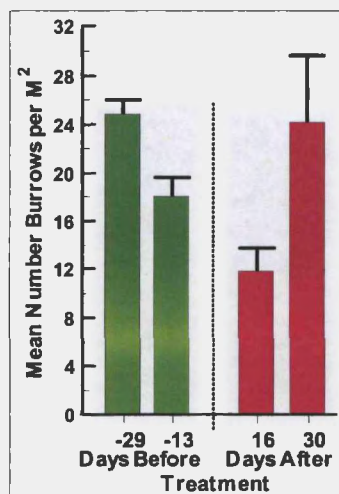
## (1) Efficacy

Average density of shrimp burrows in the 9 ac plot, treated with the granular formulation of imidacloprid at



**Figure 24** Location of 9 ac and ½ ac treatment plots on Bed A43 in North Willapa Bay and ½ ac on Bed 313 off Bay Center.

a rate of 0.5 lb a.i. per ac, were not significantly different among sample dates (Figure 25). However, samples along in-plot transects at 30 days after treatment showed that burrow density was lower in the north part of the plot where eelgrass and thick patches of dead and dying algae covered the substrate (Figure 26).

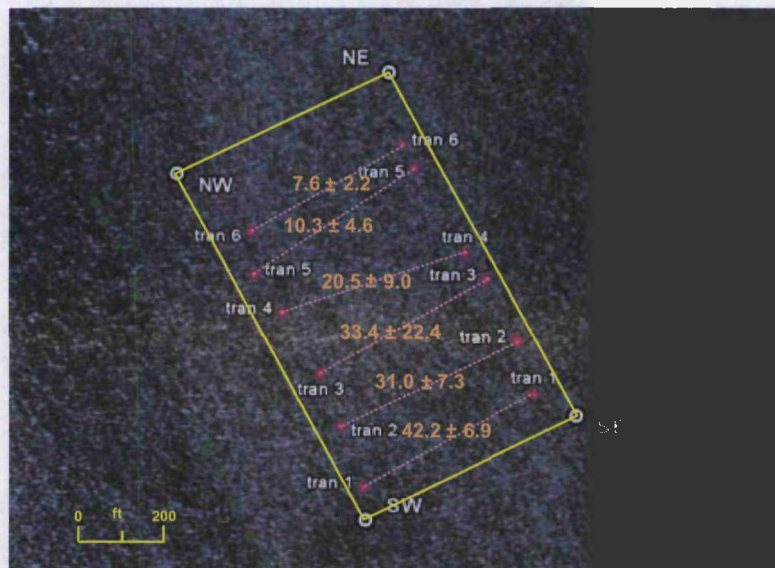


**Figure 25** Burrow density at the 9 ac bed before and after treatment with granular imidacloprid @ 0.5 lb a.i. per ac on July 21, 2009.

The number of macro-invertebrates also varied between areas with and without eelgrass (Table 26). Ghost shrimp were not always distinguished from the California giant ghost shrimp, but ~¼ of the shrimp were of the latter species.

At 4 days after treatment, burrow densities were lower, but not significantly so, in the 0.1 ac plot treated with flowable imidacloprid at 2 lb a.i. per ac than in the ½ ac bed treated with Mallet 0.5G at 0.5 lb. a.i. per ac. Burrow density in both beds was significantly lower than nearby untreated areas (Table 27).

Burrow density in the ½ ac plot on Bed 313 was significantly lower at 16 days after treatment with granular imidacloprid at 0.5 lb a.i./ac than before treatment (Table 28).



**Figure 26** Burrow density ( $\bar{x} \pm SD$  in orange) along transects in the 9 ac bed at 30 days after treatment.

**Table 26.** Average burrow count and average number of macro-invertebrates\* inside 3, 1 ft diameter rings placed in areas with and without eelgrass in the 9 ac plot at 30 days after treatment.

Substrate	burrows	macro-invertebrate		
		shrimp	<i>Mya</i> clams**	polychaete
bare mud	13.3	5.2	1.0	1.3
eelgrass	3.0	0	0	4.0

\* sampled by clam gun

\* > ¾" length

**Table 27.** Effects of two formulation /rates of imidacloprid on burrow density ( $\bar{x} \pm SE$ ) compared to an untreated check on Bed A43 at 4 days after treatment.

Formulation / Rate (lb a.i./ac)	Burrow Density *
liquid / 2.0	8.4 ± 3.3 a
granular / 0.5	16.0 ± 3.4 a
Untreated / 0	71.5 ± 8.8 b

\* Means in columns followed by the same letters are not significantly different (t-test and ANOVA; p = 0.5).

**Table 28.** Effects of granular imidacloprid applied at 0.5 lb a.i. per ac at 16 days after treatment compared to immediately before treatment in a ½ ac plot on Bed B131.

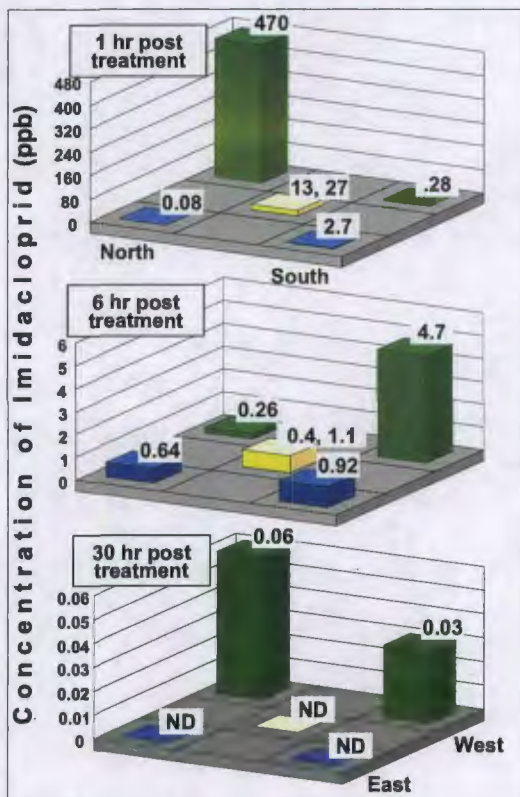
Days After Treatment	Burrow Density *
0	46.7 ± 1.8 a
16	2.2 ± 1.7 b

\* Means in columns followed by the same letters are not significantly different (t-test, p = 0.5).



Imidacloprid concentrations in water

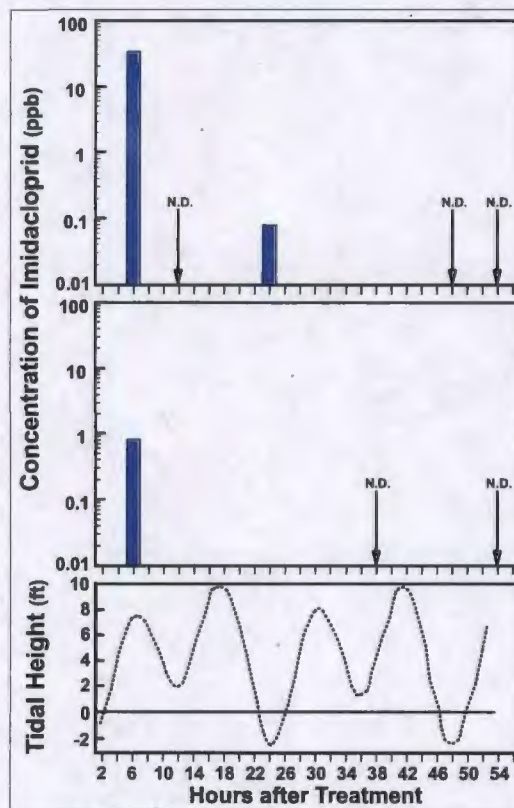
Previous studies of imidacloprid in the water following treatment of commercial scale plots showed great variation among beds in different locations and over time (2008, Figure 18). The 2009 studies showed variation within an individual bed as well as over time (Figure 27), and over time (Figure 28). In both years, concentrations declined precipitously in water directly over the bed within the first 6 hours. In 2008, imidacloprid was not detected in water sampled more than 6 hr after treatment, but low concentrations were detected at 2 of 5 within-bed sites at 30 hr after treatment in 2009. In contrast, imidacloprid was not detected in water sampled from channels at long post application intervals in 2009 (Figure 6), whereas low concentrations were detected at 48 and 72 hours after treatment in 2008.



**Figure 27** Imidacloprid concentrations in water sampled at the center and near each corner of the 9 ac A43 bed in 2009.

Imidacloprid concentrations in sediments

A very small concentration of imidacloprid was detected in sediments sampled before treatment (Table 29), likely resulting from some cross-contamination when samples were homogenized the following day, even though separate spoons were used for each sample and gloves were changed between samples. A minute concentration of imidacloprid was also found in a sample collected outside of the treated plot in substrate barren of vegetation. Imidacloprid was not detected in a sample collected outside of the plot in substrate covered in



**Figure 28** Imidacloprid concentrations in water sampled in channels near A43 and associated tidal fluctuations.

**Table 29** Concentrations of imidacloprid in sediments sampled in and outside of 9 ac treatment plot at 1 hr before or 24 hours after treatment (HAT). N.D., not detected

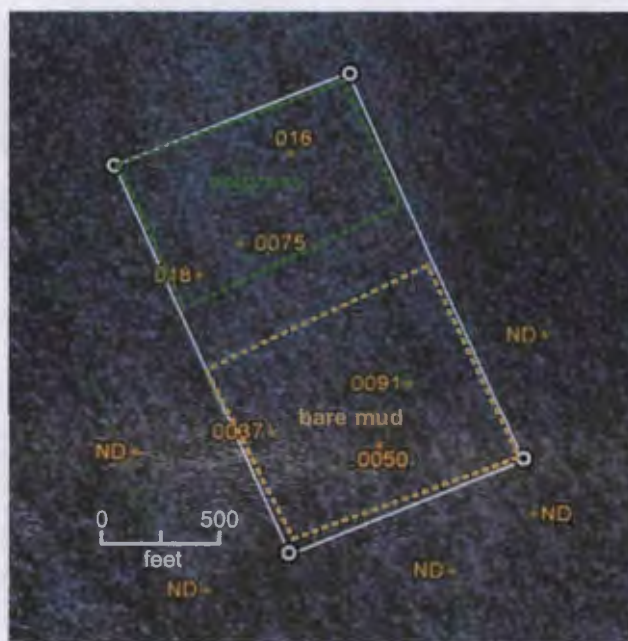
HAT	Sample Site	Substrate	Imidacloprid (ppb)
-1	In plot	bare mud	0.03
24	In plot	bare mud	.13
24	Out of plot	bare mud	.003
24	Out of plot	Eelgrass	N.D.



eelgrass. At 16 days after treatment, concentrations of imidacloprid were significantly higher in sediments sampled from the eelgrass dominated end of the plot ( $0.0138 \pm 0.0056$  ppb) than in sediments from bare mud ( $0.0059 \pm 0.0028$  ppb) (t-test,  $p = 0.05$ ). No imidacloprid was detected in sediments sampled outside the plot (Figure 29).

#### Sediment grain size and total organic carbon (TOC)

Samples from Bed A43 were comprised primarily of sediments in the 0.1 – 0.5 mm diameter range whereas samples from B313 were comprised primarily of sediments in the 0.25 – 0.1 mm diameter range and percentage TOC was 3.5 times lower (Table 6).



**Figure 29** Location and concentrations of imidacloprid of sediments sampled on and near the 9 ac treatment plot at 16 days after treatment. N.D., not detected.

Table 30. Grain Size and Total Organic Carbon (TOC) of sediments at two study sites, 2009.						
Bed	Percentage of sample among 5 ranges of grain size					TOC (%)
	(1-2 mm)	(.5 to 1 mm)	(.25 to .1mm)	(.1 to 0.5 mm)	.021 <0.05mm	
A43	0.12	1.49	6.81	89.73	1.86	1.63
B313	0.25	0.87	98.64	0.12	0.12	0.46

#### 5) Small plot trials, 2010

Objectives were to test and compare efficacy of imidacloprid formulated as a liquid (Nuprid 2F) or a granular (Mallet 0.5G) applied at 2.0 lb ai/ac or 0.5 lb ai/ac, respectively, as affected by substrate type, vegetation, and treatment timing (month of treatment). Washington State Experimental Use Permit 10009 allowed the application of both materials to a maximum of 0.99 ac each between April 16 and November 30. Actual treated acreage was 0.425 ac Nuprid 2F at 2.0 lb ai/ac (total lb ai/ac = 0.85) and 0.485 ac Mallet 0.5G at 0.5 lb ai/ac (total lb ai/ac = 0.243).

Small plot trials were conducted among four areas in Willapa Bay that varied according to general substrate type. Nuprid 2F was applied using backpack spray and Mallet using a bellygrinder during early morning maximum tides at two or more months at each area. Shrimp burrows were counted inside several  $\frac{1}{4}$  m<sup>2</sup> grids at 2 or 4 weeks after treatment. Shrimp were also counted in nearby untreated sites but only the final count is presented here.

Overall efficacy was excellent in plots treated with the liquid formulation at 2 lb ai/ac and more variable in plots treated with the granular formulation at 0.5 lb/ac (Tables 31 – 34). Both silt substrates and eelgrass cover compromised efficacy at the lower rate. The time of year for application didn't make too much difference at the high rate, but later timing compromised efficacy somewhat. Very early (April) application compromised efficacy of the low rate at the silty Cedar River site, as did very late (August) applications at the Nahcotta site.



**Table 31. Affects of imidacloprid (Mallet 0.5G at 0.5 lb ai/ac or Nuprid 2F and 2.0 lb ai/ac) on shrimp burrow density as influenced by month of treatment, type of substrate and vegetation at Leadbetter.**

Substrate / Vegetation	Month of Treatment	Burrows per m <sup>2</sup>	
		Untreated	Mallet (0.5 lb ai/ac) Nuprid (2 lb ai/ac)
Silt		16	
	April	4	0
	May	1	0
	July	4	0
	August	20	8
Dry Sand		16	
	April	4	0
	May	6	0
	July	2	1
	August	4	0
Wet Sand with Water flowing off – A		40	
	April	6	3
	May	12	2
	July	8	0
	August	20	5
Wet Sand with Water flowing off – B		64	
	April	10	0
	May	10	2
	July	4	0
	August	20	0

**Table 32. Affects of imidacloprid (Mallet 0.5G at 0.5 lb ai/ac) on shrimp burrow density as influenced by month of treatment, type of substrate and vegetation at Nahcotta.**

Month of Treatment	Burrows per m <sup>2</sup>	
	Untreated	Bare Sand Eelgrass Over Sand
	32	
May	12	17
June	8	11

**Table 33. Affects of imidacloprid (Mallet 0.5G at 0.5 lb ai/ac or Nuprid 2F at 2.0 lb ai/ac) on shrimp burrow density as influenced by month of treatment on silt substrate at Bay Center**

Month of Treatment	Burrows per m <sup>2</sup>	
	Untreated	Mallet (0.5 lb ai/ac) Nuprid (2.0 lb ai/ac)
	50	
May	14	1
June	10	1

**Table 34. Affects of imidacloprid (Mallet 0.5G at 0.5 lb ai/ac or Nuprid 2F at 2.0 lb ai/ac) on shrimp burrow density as influenced by month of treatment on silt substrate at Cedar River.**

Month of Treatment	Burrows per m <sup>2</sup>	
	Untreated	Mallet (0.5 lb ai/ac) Nuprid (2.0 lb ai/ac)
	72	
April	72	1
May	4	0
June	8	2

## 6) Large plot trials, 2010

In 2010, Federal Experimental Use Permit No. 86414-EUP-1 allowed 80 ac of Nuprid 2F at a maximum rate of 2.0 lb ai/ac and Federal Experimental Use Permit No. 86414-EUP-2 allowed application of 30 ac of Mallet 0.5G at a maximum rate of 0.5 lb ai/ac. Similar acreage limitations were cited in the Washington State EUP granted July 9 (WA 10019). Here, we describe large plot trials conducted in 2010 that featured applications of Nuprid 2F and Mallet 0.5G applied at 0.5 lb ai/ac between May 1 and October 31. However, the NPDES permit that allows applications of carbaryl on commercial oyster beds to manage burrowing shrimp also limits the treatment window for experimental applications to July 1 – October 31, so large scale treatments could not begin until July 1. Here, we describe applications of liquid (Nuprid 2F) and granular (Mallet 0.5G) imidacloprid at large plots and their effects on burrowing shrimp. Two of the trials were accompanied by assessments of non-target impact to salmonids, green sturgeon, benthic invertebrates, macro-invertebrates, and crab, plus results of samples of the water column, pore-water, and sediments for imidacloprid concentrations.

## a) Objectives

- Assess and compare the efficacy of liquid and granular formulations of imidacloprid against burrowing shrimp at a commercial scale on plots of differing vegetation density and substrate.
- Compare methods to apply granular imidacloprid.
- Assess the impact of imidacloprid to the benthic infauna
- Measure the associated concentrations of imidacloprid in the water column, pore-water, and in sediments.
- Further validate the precision and accuracy of an ELISA analytical technique compared to the standard HPLC technique.
- Survey plots following application for impact on macrobenthic organisms, especially crab and fish.
- Assess the impact of imidacloprid at the commercial scale on non-target fish.

## b) Methods

A total of 19 large plots (e.g., > 0.1 ac) on intertidal shellfish beds were treated with imidacloprid (Table 35). These comprised a total of 38.74 ac and 25.8 ac treated with Nuprid 2F and Mallet 0.5G, respectively. Total amounts of active ingredient were 62.69 lb and 13.15 lb of Nuprid 2F and Mallet 0.5G, respectively.

Two beds were located in the Oysterville growing area, 9 were in Nahcotta, 6 near the Cedar River channel, and one off Bay Center near the Palix River channel. (Figures 30, 31). All experimental beds were located at least a mile from beds treated with carbaryl as part of the standard burrowing shrimp control program. Nuprid 2F was most often applied using an ATV, but aerial applications from helicopters were made to two beds near the Cedar River channel on July 25. Mallet 0.5G was most often applied using hand held granular dispensers (bellygrinders) but at two areas, a Fimco Industries 12-volt, 2.2 cubic foot all-terrain vehicle dry material spreader with variable speed control was mounted on an ATV. The spreader was mounted on a boat for four applications. The applications from the boat, as well as two applications using the hand-held belly grinders, were made in water ~1 – 5 ft deep on the ebb tide so the grains would contact the substrate directly rather than blankets of prostrate eelgrass. The applicator was licensed and experienced.

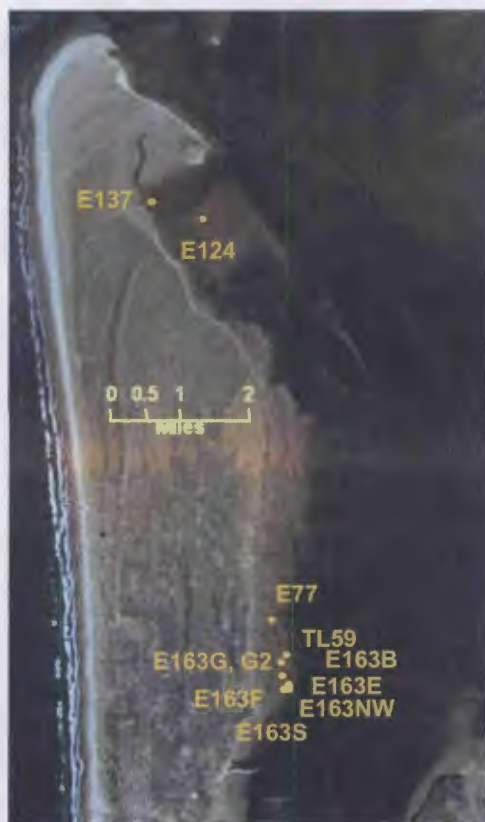
Efficacy was assessed by counting shrimp burrows inside at least fifty  $\frac{1}{4}$  m<sup>2</sup> grids along transects that crossed the plots. Non-target impact to salmonids and benthic and epibenthic infauna were sampled at two adjacent study sites in the Nahcotta area: E163B and TL59. Both sites were characterized by mostly bare sand and were exposed to similar rate and direction of tides. Nuprid 2F was applied at a rate of 2 lb ai/ac to the 10.3 ac E163B site using an ATV on July 9. Mallet 0.5G was applied to the TL59 site using the Fimco Industries granular spreader mounted on the ATV on July 10. These studies included sampling and analysis for imidacloprid in the water column, pore-water, and the sediments.



**Table 35. Characteristics for sites of large plot (>0.1 ac) experimental imidacloprid treatment, 2010.**

Material	Rate (lb ai/ac)	Bed	Growing Area	Acres / Plot	# Plots	Acres / Trt	Trt Date	App Method	Latitude <sup>a</sup>	Longitude <sup>a</sup>	Bed Type <sup>b</sup>
Mallet 0.5G	0.5	E77	Oysterville	0.25	1	0.25	12-Jul	Hand	46.531714	-124.021967	S
	0.5	E124	Oysterville	3.3	1	3.3	26-Aug	ATV	46.616550	-124.036333	S
	0.5	E137	Oysterville	0.11	2	0.22	14-Jul, 10-Aug	Hand	46.620133	-124.047250	I
	0.5	E163-G	Nahcotta	0.9	1	0.9	15-Jul	Hand	46.519920	-124.019590	S / Z. j.
	0.5	E163-G2	Nahcotta	0.9	2	1.8	15-Jul	Boat	46.517425	-124.018859	S / Z. j.
	0.5	E163-NW	Nahcotta	0.13	1	0.13	16-Jul	Hand	46.517261	-124.018234	S / Z. j.
	0.5	TL-59	Nahcotta	10	1	10	26-Jul	ATV	46.524322	-124.018732	S
	0.5	E163E	Nahcotta	0.75	2	1.5	9-Sep	Hand	46.518023	-124.018195	S vs Z. j.
	0.5	E163E	Nahcotta	0.75	2	1.5	17-Aug, 9-Sep	Hand, Boat	46.518023	-124.018195	Z. j.
	0.5	A43-G	Cedar R.	2	1	2	13-Aug	Hand	46.723489	-123.961661	I
	0.5	A43-B	Cedar R.	0.6	1	0.6	20-Oct	Boat	46.727750	-123.963722	I
	0.5	A55	Cedar R.	0.6	1	0.6	20-Oct	Boat	46.717426	-123.967600	I
	0.55	B194	Palix R	5	1	5	11-Aug	ATV	46.648467	-123.961400	S / Z. m
Nuprid 2F	2	E77	Oysterville	0.25	1	0.25	12-Jul	Hand	46.531714	-124.021967	S
	2	E137	Oysterville	0.11	2	0.22	14-Jul, 10-Aug	Hand	46.620133	-124.047250	I
	2	E163-B	Nahcotta	10.3	1	10.3	10-Jul	ATV	46.522625	-124.019827	S
	2	E163-F	Nahcotta	0.9	1	0.9	15-Jul	Hand	46.517005	-124.019496	S / Z. j.
	2	E163-S	Nahcotta	0.19	2	0.38	16-Jul	Hand	46.516945	-124.018234	S vs Z. j.
	0.5, 1, 2	E163-NW	Nahcotta	0.13	3	.39	16-Jul	Hand	46.517261	-124.018234	S / Z. j.
	2	A33	Cedar R.	3	1	3	25-Jul	Aerial	46.718060	-123.953615	I
	2	A43-N	Cedar R.	3	1	3	25-Jul	Aerial	46.729600	-123.959685	I
	2	A43-F	Cedar R.	2	1	2	13-Aug	Hand	46.722738	-123.960123	I
	0.5, 1, 1.6	B194	Palix R	5	3	15	11-Aug	ATV	46.648467	-123.961400	S / Z. m

<sup>a</sup> decimal degrees<sup>b</sup> S, Sand; I, Silt; Z. j., *Zostera japonica*; Z. m., *Zostera marina*



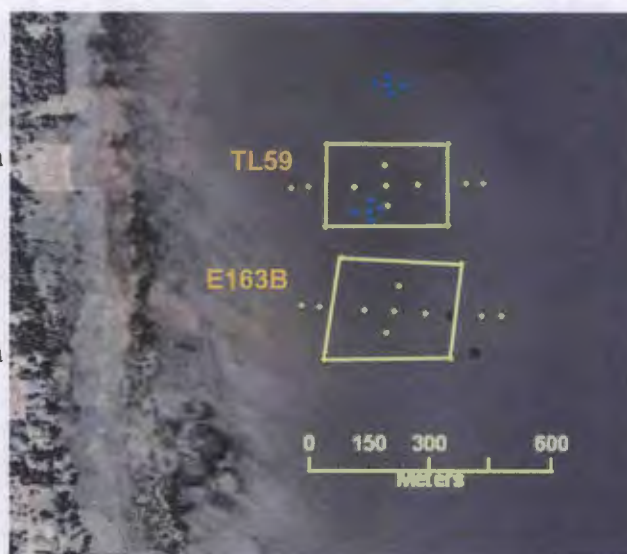
**Figure 30.** Location of experimental large plot trials in Oysterville and Nahcotta, 2010.

Water was sampled in each plot during the first tidal inundation following treatment along a transect across the bed (Figure 32). An additional sample was taken on each side of the transect 30 m from the center of the bed. Water was sampled during when it reached a depth of 15 cm (the height of the sample bottle). Additional samples were taken at the center of each bed before treatment and on several successive high tides after treatments; and in nearby off-bed channels on successive low tides.

Both the sediment and pore water were sampled on both the 10.3 ac bed treated with Nuprid 2F at 2 lb ai/ac (E163) and the 10 ac bed treated with Mallet 0.5G at 0.5 lb ai/ac. Samples were collected along the on-bed transect before treatment and at 0, 12, 24, 48, 72 hours after treatment and again at 14 and 28 days after treatment. Samples were collected using a Nalgene 500 ml bottle (7 cm diameter) with cap. The bottom of the bottle was removed and hole



**Figure 31.** Location of experimental large plot trials near the Cedar River and Palix R. channels, 2010.



**Figure 32.** Orientation of 10 ac plots E163 and TL59 and associated sample stations for water, pore-water, sediments (yellow dots), and untreated benthic infauna (blue dots). (Blue dots in TL59 correspond to treatment of E163 2 weeks before treatment of TL59. Treated benthic infauna was sampled at the same in-bed sites as the sediment samples.



was drilled into the bottle cap. The bottle was inserted into the sediment to a depth of 10 cm forcing air out of the hole. A vacuum was created by covering the hole, allowing a core sediment to be extracted from the sediment. Excess water was drained from the core and bottle and both were sealed inside a zip-lock bag. Three cores were collected and homogenized per sample point along the transect. Samples were frozen to be homogenized, extracted, and analyzed at a later date. A few sediment samples were also taken from the silty substrates at plots A43F, A43G, and A43N in the Cedar River area. Initially, only a small subset of sediment samples were analyzed to validate the accuracy and precision of the alternative, less expensive ELISA analytical technique compared to the conventional HPLC technique.

Epi-benthic and benthic infauna was sampled in plots E163B and TL59 at the same in-bed sample stations as the sediment samples at 1 day before and at 14, and 28 days after treatment. Samples were also collected at nearby untreated sites. Untreated sites for comparison with impact to E163B were sampled only at 1 day before and 14 days after treatment as they were located on TL59, which was treated 14 days after E163B. Samples were collected as previously described: a 10.2 cm internal diameter corer was inserted into the sediment to a depth of 10 cm, with each core constituting a replicate. Three replicate core samples were collected per sample site (e.g., 5 sites per sample date). Samples are currently being sorted and identification and analysis will also be as previously described.

Salmonids were captured near the 10 ac bed E163 before and after treatment with Nuprid 2F. Fish were euthanized and frozen for later analysis of brains for imidacloprid residues using an adapted ELISA technique.

### c) Results

#### (1) Efficacy

Levels of efficacy, as indicated by shrimp burrow density on treated plots and by % reduction relative to burrow density on nearby untreated plots, varied according to imidacloprid formulation and rate, method and date of application, and substrate type (sand vs silt, vegetated or not) (Table 36). Nuprid 2F consistently showed excellent efficacy, even in the two trials where it was applied at < 2.0 lb a.i./ac. Mallet 0.5G applied at 0.5 lb a.i. often bordered on being ineffective. Comparisons between the two formulation/rates were the perhaps the most rigorous at the E163B bed, where high densities of shrimp burrows were reduced to 6 per m<sup>2</sup> by Nuprid 2F but to only 16.4 per m<sup>2</sup> on the nearby and very similar TL59 bed treated with Mallet 0.5G two weeks later. At another large scale direct comparison between the two formulations at B194, Mallet 0.5G was nearly as effective as Nuprid.

#### (2) Water Samples

Preliminary results of just a few water samples from plots E163B and TL59 showed concentrations of imidacloprid declined by several orders of magnitude within 6 hours and (Table 37). Imidacloprid was not detected on-bed at any

**Table 37. Concentrations of imidacloprid (ppb) at E163 (10 ac) and TL59 (10 ac) following application of Nuprid 2F @ 2.0 lb a.i./ac and Mallet 0.5G @ 0.5 lb a.i./ac, respectively at several post-application intervals in 2010. HAT, Hours After Treatment. ND, Non-detect.**

HAT	Tide <sup>a</sup>	Site <sup>b</sup>	Nuprid 2F		Mallet 0.5G	
			On-Bed	Off-Bed	On-Bed	Off-Bed
-18	in	C	ND		ND	
0	low	Chl				ND
6	high	C	2.2		ND	
2	in	1		ND		ND
2	in	2		ND		0.29
2	in	3	31		53	
3	in	S	72		100	
3	in	C	62		120	
3	in	N	110		120	
3	in	7	13		29	
3	in	8		700		84
3	in	9		200		33
6	high	C	2.2		ND	
24	low	C	0.21			
30	high	C	0.064		0.52	
48	low	Chl		0.38		0.2
54	high	C	ND		0.067	
72	low	Chl		ND		ND
78	high	C	ND		ND	
96	low	Chl				ND
102	high	C			ND	

<sup>a</sup> in, inundating

<sup>b</sup> C, Center; S, South of center; N, North of center

post-application interval > 6 hours after treatment with Mallet 0.5G at 0.5 lb ai/ac, but was detected at very low levels on the bed treated with Nuprid 2F at 2 lb ai/ac at 24 and 48 hrs after treatment.

**Table 36. Shrimp burrow densities ( $\bar{x} \pm \text{S.E.}$ ) on large (>0.1 ac) plots treated with Mallet 0.5G or Nuprid 2F and on nearby untreated plots at 2 weeks after treatment (WAT). % Reduced is treated compared to untreated densities. Bolded values indicate questionable suppression.**

Material	Rate (lb ai/ac)	Bed	Growing Area	Acres / Plot	Trt Date	App Method	Bed Type <sup>b</sup>	Burrow Density (# / m <sup>2</sup> )			
								estimated pre-	at 2 WAT		% Reduced
									Treated	Untreated	
Mallet 0.5G	2.0	E124	Oysterville	3.30	26-Aug	Boat	S	40-60	2.4 ± 0.8	80.8 ± 2.0	97.0
	0.5	E137	Oysterville	0.11	14-Jul	Hand	I	20	3.2 ± 0.8	15.2 ± 2.0	78.9
	0.5	E137	Oysterville	0.11	10-Aug	Hand	I	20	3.2 ± 0.4	16.0 ± 2.0	80.0
	0.5	E163-G	Nahcotta	0.90	15-Jul	Hand in water	S / Z. j.	25-30	<b>15.6 ± 1.6</b>	36.0 ± 1.2	<b>56.7</b>
	0.5	E163-G	Nahcotta	1.80	15-Jul	Boat	S / Z. j.	25-30	10.8 ± 0.8	36.0 ± 1.2	70.0
	0.5	E163-N	Nahcotta	0.13	16-Jul	Hand	S / Z. j.	25-30	0	30.8 ± 1.6	100
	0.5	TL-59	Nahcotta	10.00	26-Jul	ATV	S	30-40	<b>16.4 ± 3.2</b>	61.2 ± 9.2	73.2
	0.5	E163E	Nahcotta	0.75	9-Sep	Hand	Z. j.	30-40	8.8 ± 1.2	26.4 ± 1.2	66.7
	0.5	E163E	Nahcotta	0.75	9-Sep	Hand	S	30-40	<b>18.4 ± 1.2</b>	53.2 ± 6.8	<b>65.4</b>
	0.5	E163E	Nahcotta	0.75	17-Aug	Boat	S	30-40	<b>33.6 ± 1.6</b>	52.0 ± 2.0	<b>35.4</b>
	0.5	E163E	Nahcotta	0.75	9-Sep	Hand	S	30-40	<b>13.6 ± 1.6</b>	49.6 ± 1.6	<b>72.6</b>
	0.5	A43-G	Cedar R.	2.00	13-Aug	Hand	I	20-40	8.8 ± 1.2	79.6 ± 2.0	88.9
	0.5	A43-B	Cedar R.	0.60	20-Oct	Boat	I	20-40	<b>15.2 ± 8.8</b>	30.0 ± 1.6	<b>49.3</b>
	0.5	A55	Cedar R.	0.60	20-Oct	Boat	S / I	20-40	0.8 ± 0.4	6.4 ± 0.8	87.5
	0.55	B194	Palix R.	5.00	11-Aug	ATV	S	60	1.6 ± 1.6	52.0 ± 1.2	96.9
Nuprid 2F	2.0	E137	Oysterville	0.11	14-Jul	Hand	I	20	2.0 ± 0.4	14.8 ± 2.0	86.5
	2.0	E137	Oysterville	0.11	10-Aug	Hand	I	20	1.2 ± 0.4	14.4 ± 1.2	91.7
	2.0	E124	Oysterville	3.30	26-Aug	ATV	S	40-60	0	80.8 ± 2.0	100
	2.0	E163-B	Nahcotta	10.30	10-Jul	ATV	S	30-40	6.0 ± 0.8	58.4 ± 8.0	83.3
	2.0	E163-F	Nahcotta	0.90	15-Jul	Hand	S / Z. j.	25-30	2.8 ± 0.4	36.0 ± 1.2	94.7
	2.0	E163-S	Nahcotta	0.19	16-Jul	Hand	S	25-30	4.4 ± 0.8	53.2 ± 6.8	83.3
	2.0	E163-S	Nahcotta	0.19	16-Jul	Hand	Z. j.	25-30	0	26.4 ± 1.2	100
	0.5	E163-	Nahcotta	0.13	16-Jul	Hand	S / Z. j.	25-30	0	30.8 ± 1.6	100
	1.0	E163-	Nahcotta	0.13	16-Jul	Hand	S / Z. j.	25-30	1.6 ± 0.8	30.8 ± 1.6	94.8
	2.0	E163-	Nahcotta	0.13	16-Jul	Hand	S / Z. j.	25-30	0	30.8 ± 1.6	100
	2.0	A33	Cedar R.	3.00	25-Jul	Air	I	20-40	0.8 ± 0.4	79.6 ± 2.0	99.0
	2.0	A43-N	Cedar R.	3.00	25-Jul	Air	I	20-40	1.2 ± 0.4	73.2 ± 2.0	98.4
	2.0	A43-F	Cedar R.	2.00	13-Aug	Hand	I	20-40	2.0 ± 0.4	79.6 ± 2.0	97.5
	0.5	B194	Palix R.	5.00	11-Aug	ATV	S	60	0.4 ± 0.4	50.0 ± 1.6	99.2
	1.0	B194	Palix R.	5.00	11-Aug	ATV	S	60	0	50.0 ± 1.6	100
	1.6	B194	Palix R.	5.00	11-Aug	ATV	S	60	0	50.0 ± 1.6	100

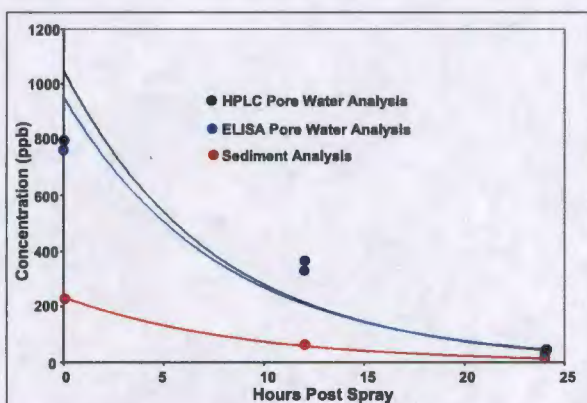
<sup>a</sup> decimal degrees

<sup>b</sup> S, Sand; I, Silt; Z. j., *Zostera japonica*; Z. m., *Zostera marina*

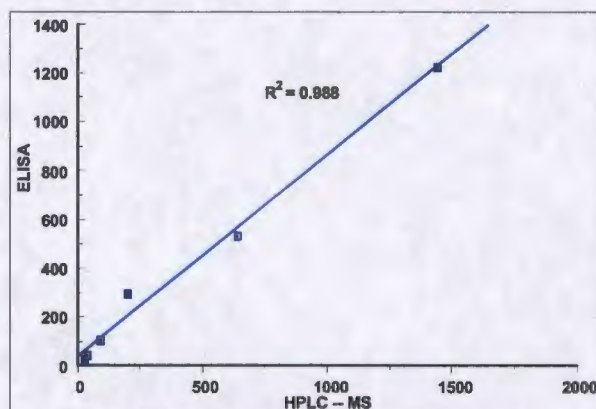


## (3) Pore water and sediment samples

Preliminary results of the two on-bed pore water samples from both beds (north and south mid-bed transect points) showed exponential declines from samples taken immediately after application and at 6, 12, and 24 hr after treatment (Figure 33). Concentrations of imidacloprid in sediments (ppb, dry weight) also declined over time. Parallel analyses for concentrations of imidacloprid in pore water using both the standard technique, EPA 8321B (HPLC-MS) and a detection limit of 10 ppb, and the alternative, less expensive ELISA technique (detection limit 0.07 ppb) showed strong agreement (Figure 34).



**Figure 33.** Imidacloprid concentrations in water as analyzed by HPLC and ELISA and in sediments.



**Figure 34.** Alternative ELISAs vs standard HPLC analysis for imidacloprid in pore water.

## (4) Observed mortality to macro-invertebrates and crabs

Some dead or crab in tentanous shock were observed in large plots. No other dead macro-invertebrates were observed (Table 38).

**Table 38.** Estimated number\* of affected (in tetany or dead) crab per ac at 2 days after treatment with imidacloprid (Mallet 0.5G, Nuprid 2F) or carbaryl (Sevin) in 16 large plots.

Material	Bed	Rate (lb ai/ac)	Acres Treated	Trt Date	Affected Crab (#/ac)
Mallet 0.5G	E124	2	3.3	26-Aug	1
	E163-G / G2	0.5	1.8	15-Jul	13
	E163-NW	0.5	0.13	16-Jul	0
	TL-59	0.5	10	26-Jul	1
	E163E	0.5	2.25	9-Sep	0
	E163E	0.5	0.75	17-Aug	0
	A43-G	0.5	2	13-Aug	1
Nuprid 2F	E137	2	0.11	14-Jul	0
	E137	2	0.11	10-Aug	0
	E163-B	2	10.3	10-Jul	36
	E163-F	2	0.9	15-Jul	1
	E163S	2	0.38	9-Sep	0
	E163-NW	0.5, 1, 2	0.39	16-Jul	0
	A43-N	2	3	25-Jul	20 *
Sevin	A43-F	2	2	13-Aug	1
	E1	8	7.1	10-Jul	111

\* Dismembered or parts of crab were counted as whole. Many single claws were observed at A43-N, which may have inflated the estimate.

## (5) Epi-benthic and benthic infauna samples

Samples are currently being sorted from debris.



**F) Proposed Experimental Program****1) Qualifications and Identifications of Participants****a) Researchers***Dr. Kim Patten*

Professor and Extension Specialist

Washington State University

Long Beach Extension Center

Longbeach, WA

**Degrees:**

Undergraduate: 1977

University of California

Davis, CA

Masters: 1980

Iowa State University

Ames, IA

Ph. D.: 1984

Washington State University

Pullman, WA

**Areas of active research:** Dr. Patten is Station Director at the Long Beach Extension Center, where he works in cranberry, shellfish, and invasive weed control.

**Selected Recent Publications:**

Patten, K and C. O'Casey 2007. Use of Willapa Bay, Washington, by shorebirds and waterfowl after *Spartina* control efforts. *J. Field Ornithol.* 78(4):395-400

Patten, K. 2006. Review of Clearcast (Imazamox) Aquatic EUP and research results for the western U.S. *Proceedings of Aquatic Plant Management Society*. August, 2006.

Patten, K. 2006. Parrotfeather milfoil (*Myriophyllum aquaticum*) and water primrose (*Ludwigia hexapetala*) control with herbicides. *Proc. of the Western Aquatic Plant Management Society*. March, 2006

Patten, K. 2006. Design and evaluate subsurface chemical delivery systems and deep penetrating harrow for management of burrowing shrimp populations. *Shellfish Journal*.

Patten, K. 2005. Burrowing shrimp control. *Pacific Coast Shellfish Grower Conference* (abstract)

Patten, K. 2005. Watershed mapping of cranberry farms BMPs to reduce surface water pesticides. *WSU Extension Conference*.

Patten, K. 2005. Invasive *Spartina* in west coast estuaries. *The Journal of Marine Education* 21:27-31.

Patten, K. 2003. Persistence and non-target impact of imazapyr associated with smooth cordgrass control in an estuary. *Journal of Aquatic Plant Management* 41:1-5.

Hedge, P., L. Kriwoken, and K. Patten. 2003. A review of *Spartina* management in Washington, USA. *J. Aquatic Plant Management* 41:82-90.

Patten, K. 2003. Eradicating *Spartina* and restoring affected mudflats using herbicides, new application technologies and supplemental mechanical methods. *Abstracts in Invasive Plants in Natural and Managed Systems: 7th International Conference on the Ecology and Management of Alien Plant Invasions*. October 2003. Ft. Lauderdale, FL. (abstract).

***Dr. Christian Grue***

Associate Professor, Aquatic &amp; Fishery Sciences

Unit Leader, Washington Cooperative Fish and Wildlife Research Unit

University of Washington

Seattle, WA 98195

**Degrees:**

Undergraduate: 1972

University of California

UC Santa Barbara, CA

Masters: 1977

Northern Arizona University

Flagstaff, AZ

Ph. D.: 1977

Texas A&amp;M University

College Station, WA

**Duties and Research Interests:** Dr. Grue is leader of the Washington Cooperative Fish and Wildlife Research Unit. Dr. Grue's research and that of his graduate students at the University of Washington has focused on the efficacy and non-target effects of chemical and biological pest control within aquatic environments with an emphasis in Washington State and the Pacific Northwest. Recent studies include comparisons in the toxicity among active ingredients, formulated products and tank mixes (end products), effects of *Bti* control of mosquitoes on aquatic invertebrate communities, and the effects of pesticides in surface waters on the survival and reproduction of salmonids. He teaches a class in fish and wildlife toxicology. Dr. Grue is an active member the Society of Environmental Toxicology and Chemistry and the Wildlife Society and frequently serves on advisory panels dealing with pesticides and other environmental contaminants. He has recently served on FIFRA Science



Advisory Panels, the Five-year Review Committee for the USGS's Contaminant Biology Program, and the Editorial Board of the Bulletin of Environmental Contamination and Toxicology, and was recently appointed to the External Advisory Group for the Washington Department of Ecology dealing with the agency's permit for aquatic weed control and eradication.

Selected Recent Publications:

- Grue, C.E., S.C. Gardner and P.L. Gibert. 2002. On the significance of pollutant-induced alterations in the behavior of fish and wildlife. Chapter 1 (pages 1-90) in G. Dell'Omo (ed.) Behavioural Ecotoxicology, John Wiley & Sons, Ltd., West Sussex, UK.
- Major, W.W., III, C.E. Grue, S.C. Gardner and J.M. Grassley. 2003. Concentrations of glyphosate and AMPA in sediment following application of Rodeo® to control smooth cordgrass in Willapa Bay, Washington. Bulletin of Environmental Contamination and Toxicology 71:912-918.
- Curran, C.A., J.M. Grassley and C.E. Grue. 2004. Toxicity of R-11® surfactant to juvenile rainbow trout: Does size matter? Bulletin of Environmental Contamination and Toxicology 72:401-408.
- Smith B.C., C.A. Curran, K.W. Brown, J.L. Cabarrus, J.B. Gown, J.K. McIntyre, E.E. Moreland, V.L. Wong, J.M. Grassley and C.E. Grue. 2004. Toxicity of four surfactants to juvenile rainbow trout: Implications for over-water use. Bulletin of Environmental Contamination and Toxicology 72:647-654.
- Getsinger, K.D., M.D. Netherland, C.E. Grue and T.J. Koschnick. 2008. Improvements in the use of aquatic herbicides and establishment of future research directions. Journal of Aquatic Plant Management (In press).
- Grue, C.E., C.A. Curran J.L. Cabarrus S.C. Gardner, N. Spang, J.M. Grassley, B.C. Smith, and K.A. King. Active ingredients, formulations and tank mixes: What should be regulated? Integrated Environmental Assessment and Management (In external review).
- Tamayo, M., C.E. Grue and L.L. Conquest. Response of wetland invertebrates to mosquito control. Journal of Applied Ecology (External review completed, submission December 2007).
- King, K.A., W.L. Madden, C.A. Curran, R.A. Battin Jr, C.T. Elfes, S.R. Frame, J. Kim, M.T. McDaniel, V.A. Pelekis, M.R. Sternberg, J.M. Grassley, and C.E. Grue. Brain AChE inhibition in juvenile rainbow trout exposed to pesticide mixtures within urban streams in western Washington: Non-additive effects. Bulletin of Environmental Contamination and Toxicology (Ready for external review).
- Grue, C.E., C.T. Elfes, S. Booth, B.R. Dumbauld, A.S. Felsot, N.C. Overman, J.M. Grassley, and W.W. Major III. Commentary — Behavioral impairment and increased predation mortality in cutthroat trout exposed to carbaryl: Leaps of faith and pious hopes. Marine Ecology Progress Series (Submission December 2007).

*Dr. Vince Hebert*

Laboratory Research Director,  
Food and Environmental Quality Laboratory  
Washington State University-Tri Cities  
Food and Environmental Quality Lab  
Richland, WA

Degrees:

Undergraduate: 1983	Masters: 1987	Ph. D.: 1999
Humboldt State University	University of Nevada	University of Nevada
Arcata, CA	Reno, NV	Reno, NV

Areas of active research: 1) developing analytical methods for assessing specific biomarkers useful for monitoring pesticide exposures to sensitive subpopulations in agricultural communities, 2) the development of field air -sampling methods and volatilization chamber system design for assessing fumigants, pesticides, and semiochemicals useful in codling moth mating disruption, 3) characterizing/isolating bioactive plant volatile emissions from insect herbivory that may prove useful in enhancing conservation biological control in cropping systems, and 4) chemically assessing sublethal concentrations of pesticides in surface waters that can have neurobehavioral effects on salmonids. A principle responsibility is to administer over a state-mandated food and environmental regulatory science facility that conducts studies under federal 40CFR Part 160 Good Laboratory Practices (GLP). This program houses an independent quality assurance unit and GLP Laboratory Coordinator to assure federal compliance.

Selected Recent Publications:

- Hebert VR and Miller GC. Understanding the tropospheric fate of agricultural pesticides, in Reviews of Environmental Contamination and Toxicology, ed. G. Ware, Vol. 181 pp 1-36 (2004).

- Woodrow J, Hebert VR, LeNoir J. "Monitoring Of Agrochemical Residues In Air." in "Handbook of Residue Analytical Methods for Agrochemical Residues" (P. Lee ed., two volume series) John Wiley & Sons. pp. 908-935 (2003).
- Merriman J, Hebert VR Methyl Isothiocyanate Residential Community Air Assessment; South Franklin County, Washington. Bull of Environ Contam and Toxicol. In press (Jan 2007)
- Hebert, VR. Understanding the tropospheric transport and fate of semivolatile pest management chemicals. In: Environmental Fate and Safety Management of Agrochemicals ACS Symposium Book Series 899, ed. JM Clark, pp 70-82 (2005).
- Hebert, VR, Hoonhout C, Miller GC. Reactivity of certain gas-phase organophosphorus insecticides toward hydroxyl radicals at elevated air temperatures. J. Agric. Food. Chem, Vol. 48: (2000): 1922-1928.
- Hebert, VR, E Tomaszewska, J. F. Brunner, V. P. Jones, and M. Doerr. Evaluating the pheromone release rate characteristic of commercial mating disruption devices. In Crop Protection Products for Organic Agriculture. Environmental, Health, and Efficacy Assessment. Felsot, A.S., K. D. Racke (ed.); Am. Chem. Soc., Symposium Series 947, Am. Chem. Soc., Washington, DC. pp. 144-157 (2006).
- Weppner, S, Elgethun K, Lu C, Hebert VR\*, Yost M, Fenske R. The Washington aerial spray drift study: Children's exposure to methamidophos in an agricultural community following fixed-wing aircraft application J. Expos. Anal. Environ. Epidem 16: 387-396 (2006).

*Dr. Allan Felsot*

Professor and Extension Specialist  
Entomology and Environmental Toxicology  
Washington State University-Tri Cities  
Food and Environmental Quality Lab  
Richland, WA

Degrees:

Undergraduate: 1972	Masters: 1974	Ph. D.: 1978
Tulane University	University of Florida	Iowa State University
New Orleans, LA	Gainesville, FL	Ames, IA

Research and Extension Interests: Hazard assessments of transgenic crops, pesticide drift and buffer zone design, reduction of insecticide application rates using new sprayer technologies, enhanced biodegradation of pesticides, remediation of pesticide waste in soil, best management practices for controlling agrochemical movement to surface and ground water, analytical chemistry of pesticide residues in soil, water, and food, pesticide toxicology, regulations, and risk communication. He teaches a graduate course entitled "Applied Environmental Toxicology." He also team teaches the course, "Pesticides: Toxicology and Modes of Action."

Recent Publications:

- Felsot, A. S. 2004. Establishing buffers: Protocols and toxicological benchmarks, Proc. International Conference on Pesticide Application for Drift Management. Oct 27-29, Waikoloa, HI. pp. 199-203.
- Felsot, A. S. 2004. Impact of U.S. court cases on application technology, Proc. International Conference on Pesticide Application for Drift Management. Oct 27-29, 2004, Waikoloa, HI. pp. 53-58.
- Felsot, A. S. 2004. Is the content of disease-reducing phytochemicals influenced by certified organic crop production practices? Paper no. 21, 228th National Mtg. American Chemical Society (PICOGRAM Issue no. 67, p. 55), Aug 22-26, 2004. Philadelphia, PA.
- Ramaprasad, J., M.-Y. Tsai, K. Elgethun, V. R. Hebert, A. Felsot, M. G. Yost, R. A. Fenske. 2004. The Washington aerial spray drift study: assessment of off-target organophosphorus insecticide atmospheric movement by plant surface volatilization. Atmospheric Environment 38:5703-5713.
- Felsot, A. S., 2004. No-spray buffer zones for the ag/urban interface: derivation using drift modeling and toxicologically relevant benchmarks (26 MB \*.pdf). Paper no. 85, 227th National Mtg. American Chemical Society (PICOGRAM Issue no. 66, p. 68), Mar 28-Apr 1, 2004. Anaheim Calif.

**b) Consultants***Dr. Alan Schreiber*

President, Agriculture Development Group, Inc., Pasco Washington  
Administrator - Washington State Commission on Pesticide Registration  
Executive Director - Washington Asparagus Commission

Degrees:

Undergraduate: 1984	Masters: 1987	Ph. D.: 1991
Northeast Missouri St. Univ.	University of Missouri	University of Missouri



Kirkville, MO

Columbia, MO

Columbia, MO

Research and Extension Interests: For the Ag Development Group, Dr. Schreiber consults on environmental, pesticide, pest management and Food Quality Protection Act issues for grower groups, governmental organizations and agribusiness's and conducts research on more than 30 crops on a 100 acre research farm. For the WSCPR, a state governmental entity dedicated to support of activities related to pesticide registration and pest management, Dr. Schreiber manages a \$0.9 million budget and interacts with all commodity and pest management groups, pest management researcher and extension specialist in Washington. Prior to these positions, Dr. Schreiber was Assistant Professor for the Department of Entomology, Washington State University, and before that, Entomologist for the USEPA/Office of Pesticide Programs/Biological and Economic Analysis Division

Honors and Awards:

Outstanding Service Award to U.S. Potato Industry, National Potato Council, 2002  
Entomological Society of America, Excellence in Extension nominee, 1997  
WSU Outstanding Extension Scientist, Department of Entomology nominee,  
1997 Oregon/Washington Asparagus Growers Assn. "Friend of the Industry Award,"  
1996 Columbia Basin Vegetable Seed Association Outstanding Service Award, 1995

*Dr. Steven Booth*

PSI / WGHOGA

120 State St. NE #142

Olympia, WA 98501

Degrees:

Undergraduate: 1975

University of Iowa

Iowa City, IA

Masters: 1982

Western Washington University

Bellingham, WA

Ph. D.: 1992

Oregon State University

Corvallis, OR

Research and Extension Interests: As the IPM Coordinator for the Willapa Bay / Grays Harbor Oyster Growers Association, Dr. Booth assists in the development and implementation of a variety of chemical, biological, and mechanical tactics for the control of burrowing shrimp. He has writes grant proposals to fund the IPM program and reports that describe its progress. Prior to his current position, Dr. Booth has developed IPM tactics featuring biorational pesticides, insect parasitic nematodes and fungi, and beneficial insects.

Recent Publications:

Booth, S.R., Drummond, F. and E. Groden. 2007 Special considerations for application and evaluation of entomopathogens in specific systems: Small fruits. *in* Field Manual of Techniques for the Use and Evaluation of Entomopathogens, 2<sup>nd</sup> Edition. [L. Lacey and H. Kaya, eds., Ch. VII.12. Kluwer Press. pp 583 – 598.

Dumbauld, B.R., Booth, S.R., Cheney, D., Suhrbier, A., and H. Beltran. 2006. An integrated pest management program for burrowing shrimp control in oyster aquaculture. *Aquaculture*.261: 976-992.

Booth, S.R., Tanigoshi, L.K., and Shanks, C., Jr. 2002. Evaluation of entomopathogenic nematodes to manage root weevil larvae in Washington state cranberry, strawberry, and red raspberry. *Env. Entomol.* 31:895-902.

Booth, S.R., Tanigoshi, L.K., and I. Dewes. 2000. Potential of a dried mycelium formulation of an indigenous strain of *Metarhizium anisopliae* against subterranean pests of cranberry. *Biocontrol Science and Technology* 10:659-668.

Booth, S.R. and C.H. Shanks. 1998. Potential of a dried rice/mycelium formulation of entomopathogenic fungi to suppress subterranean pests in small fruits. *Biocontrol Science and Technology*. 8:197-206.

**c) Grower Cooperators – members of WGHOGA who own acreage allotments**

<i>Kristi Ballo</i> Brady's Oysters 3714 Oyster Pl. E. Aberdeen, WA 98520	<i>Nick Jambor</i> Ekone Oyster Co. 29 Holtz Road South Bend, WA 98586	<i>Brian Sheldon</i> Northern Oyster Company PO Box 1039 Ocean Park, WA 98640
<i>Leonard Bennett</i> R&B Oyster Co P O Box 309 Bay Center, WA 98586	<i>James Kemmer</i> Long Island Oyster PO Box 1054 Long Beach, WA 98631	<i>Jerry Swan</i> Grass Creek Oyster Co 1975 Lakemoore Pl SW Olympia, WA 98512
<i>Warren Cowell</i> Willapa Bay Shellfish, Inc. P O Box 43 Ocean Park, WA 98640	<i>Tim Morris</i> Coast Seafoods Box 166 South Bend, WA 98586	<i>Bill Taylor / Eric Hall</i> Taylor Shellfish Co., Inc. SE 130 Lynch Road Shelton, WA 98584
<i>Dan Driscoll</i> Oysterville Seafarms P O Box 6 Oysterville, WA 98641	<i>Dave Nisbet</i> Nisbet Oyster Co. PO Box 338 Bay Center, WA 98527	<i>Dennis Tufts</i> Wilson Oyster Co. PO Box 236 Ocean Park, WA 98640
<i>Don Gillies</i> Stony Point Oyster Co. L.L.C. 6931 US Hwy 101 South Bend, WA 98586	<i>Phil Olsen</i> Olsen & Son Oyster Co. PO Box 212 South Bend, WA 98586	<i>Fritz Wiegardt</i> Wiegardt & Sons P O Box 309 Ocean Park, WA 98640
<i>John Heckes</i> Heckes Clam Co P O Box 1657 Ocean Park, WA 98640	<i>Eric Petit</i> Willapa Fish & Oyster PO Box 524 South Bend, WA 98586	<i>Dr. Richard Wilson</i> Bay Center Mariculture P O Box 356 Bay Center, WA 98586
<i>David Hollingsworth</i> Markham Oyster Inc. 20 Old Westport Road. Aberdeen, WA 98520		

**2) Locations, acreage to be treated**

All areas to be treated lie within the 4,250 intertidal acreage of Willapa Bay and Grays Harbor (4250 ac (Feldman et al. 2000) and 7,500 ac (<http://graysharbor.fws.gov>), respectively). Most of the 35,000 commercial acreage (BSCC 1992) lie several hundred meters from land and human habitation. A maximum acreage of 120 intertidal ac will be treated with imidacloprid. Treatments will feature liquid soluble concentrate imidacloprid (Nuprid 2F; NuFarm America, Inc.) applied at 2.0, 1.0, and 0.5 lb a.i./ac and 0.5% granular imidacloprid (Mallet 0.5 G; NuFarm, Inc.) applied at 0.5 lb a.i./ac. The exact location and size of experimental plots cannot be determined until spring, 2011, but the tentative treatment schedule calls for mostly 5 ac plots of each rate / formulation combination plus four 10 ac plots of Nuprid 2F applied at 2.0 lb (Table 39). The 10 acre plot and two of the 5 ac plots in the Cedar River area will be used to study non-target affects to epi-benthic and benthic infauna as well as the fate and transport of imidacloprid following applications of Nuprid 2F at 2 lb ai/ac to the 10 ac plots and Mallet 0.5G at 0.5 lb ai/ac to the 5 ac plots by boat. The time necessary to apply the granular material by boat currently limits plot size to 5 ac. The 10 ac applications of each material to silty Cedar River sediments will be comparable to similar 10 ac treatments of each material to sandy Nahcotta sediments in 2010. Final bed sites will selected based on based on density of burrowing shrimp, substrate type, grower cooperation, ease of access, size, proximity to beds targeted for carbaryl application and proximity to untreated areas.



**Details of the Proposed Program**

Beds will likely be distributed among 5 general treatment areas in Willapa Bay (Figure 35). It is not possible to compare all formulation / rate treatments at each study site (e.g., a factorial experimental design) due to area limitations and a desire to minimize potential impact. Instead, selected treatment combinations will be more likely be compared in pairs or triplicates. Nuprid 2 F will be applied mostly using an ATV carrying pump and booms. At least one application of the Nuprid 2F will be made aurally using helicopters, as in the conventional carbaryl-based shrimp management program. Mallet 0.5G will be applied at 0.5 lb ai/ac using conventional ground-based granular dispensers (e.g., belly grinders), either by hand or mounted on an ATV or boat.

Several applications will be made at early season dates as thick blankets of eelgrass and algae that develop during mid to late season have hampered efficacy in trials conducted in previous years.

**Figure 35.** Five treatment areas in Willapa Bay.

**Table 39. Tentative 2011 experimental trials of imidacloprid (Nuprid 2F and Mallet 0.5G)**

Major Objectives	Timing	Sediment	Area	Application Method	Plot Size (ac)	Material	Rate (lb ai/ac)
Seasonal / Vegetational Affects	April	Sand	Nahcotta	ATV	5.0	Mallet 0.5G	0.5
Seasonal / Vegetational Affects	May	Sand	Nahcotta	ATV	5.0	Mallet 0.5G	0.5
Seasonal / Vegetational Affects	June	Sand	Nahcotta	ATV	5.0	Mallet 0.5G	0.5
Seasonal / Vegetational Affects	April	Sand	Nahcotta	ATV	5.0	Nuprid 2F	2.0
Seasonal / Vegetational Affects	May	Sand	Nahcotta	ATV	5.0	Nuprid 2F	2.0
Seasonal / Vegetational Affects	June	Sand	Nahcotta	ATV	5.0	Nuprid 2F	2.0
Application method, Substrate Affects	July	Sand	Nahcotta	Aerial	10	Nuprid 2F	2.0
Application method, Substrate Affects, Seasonal Affects	June	Sand/Silt	Bay Center	ATV	5.0	Nuprid 2F	2.0
Application method, Substrate Affects, Seasonal Affects	June	Sand/Silt	Bay Center	Boat	5.0	Mallet 0.5G	0.5
Application method, Substrate Affects, Seasonal Affects	July	Sand/Silt	Bay Center	ATV	5.0	Nuprid 2F	2.0
Application method, Substrate Affects, Seasonal Affects	July	Sand/Silt	Bay Center	Boat	5.0	Mallet 0.5G	0.5
Application method, Substrate Affects	July	Sand/Silt	Bay Center	Aerial	10	Nuprid 2F	2.0
Application method, Substrate Affects, Seasonal Affects	April	Sand/Silt	Leadbetter	ATV or Boat	5.0	Mallet 0.5G	0.5
Application method, Substrate Affects, Seasonal Affects	May	Sand/Silt	Leadbetter	ATV or Boat	5.0	Mallet 0.5G	0.5
Application method, Substrate Affects, Seasonal Affects	May	Sand/Silt	Leadbetter	ATV	5.0	Nuprid 2F	2.0
Application method, Substrate Affects, Seasonal Affects	June	Sand/Silt	Leadbetter	ATV	5.0	Nuprid 2F	2.0
Application method, Substrate Affects, Seasonal Affects	June	Sand/Silt	Leadbetter	ATV or Boat	5.0	Mallet 0.5G	0.5
Application method, Substrate Affects, Seasonal Affects	July	Sand/Silt	Leadbetter	ATV or Boat	5.0	Mallet 0.5G	0.5
Application method, Substrate Affects, Seasonal Affects	July	Sand/Silt	Leadbetter	ATV	5.0	Nuprid 2F	2.0
Application method, Substrate Affects, Seasonal Affects	August	Sand/Silt	Leadbetter	ATV or Boat	5.0	Mallet 0.5G	0.5
Application method, Substrate Affects, Seasonal Affects	August	Sand/Silt	Leadbetter	ATV	5.0	Nuprid 2F	2.0
Application method, Substrate Affects	July	Sand/Silt	Stoney Pt / Pine Isl	Aerial	10	Nuprid 2F	2.0
Application method, Fate & Transport, Impact to crab & benthic in-fauna	July	Silt	Cedar R	Boat	5.0	Mallet 0.5G	0.5
Application method, Fate & Transport, Impact to crab & benthic in-fauna	July	Silt	Cedar R	Boat	5.0	Mallet 0.5G	0.5
Application method, Fate & Transport, Impact to crab & benthic in-fauna	July	Silt	Cedar R	Aerial	10.0	Nuprid 2F	2.0
Substrate Affects	July	Silt	Cedar R	ATV	5.0	Nuprid 2F	2.0



### Efficacy

Efficacy will be judged primarily by comparing shrimp burrow counts taken before treatment and at several post treatment intervals (~4 – 8 weeks and, pending results, 11 months after treatment). On commercial beds, the length of the interval before sampling will also depend on when seed is planted. Walking on newly planted seed will substantially damage the crop. Efficacy on each bed will also be eventually and ultimately be judged by yield.

### Fate and Transport of Imidacloprid

The fate and transport of imidacloprid in estuarine water following experimental applications will be investigated based on water sampled from both the water column and pore water, and from samples of the sediments. Samples in 2010 were primarily from sandy sites near Nahcotta with a few samples from silty sites near the Cedar River. In 2011, we propose to focus on sites at the Cedar River with a few comparable samples at the Nahcotta site. Specific sample stations at these sites have yet to be determined pending results from the 2010 trials, but in general, samples will be taken at the site of application, at increasing distances from the application site, and at increasing post-application intervals.

On-bed water column samples will be collected on the first incoming tide following treatment when depth reaches 10 cm, and on the first, third, and fifth high tides after treatment (i.e., 6, 31, 55, and 80 hr after treatment). Samples will also be collected in channels near treatment sites on the same high tides following treatment and on the low tides at 24, 49, and 74 hr after treatment. Water column samples at high tides will be collected at 1 m beneath the surface using a Niskin sampler and at low tides at depths of at least 10 cm by hand (grab samples).

Depending on results of 2010 studies, pore water and sediments will likely be sampled at stations and times similar to those used in 2010: along a transect that crosses the bed parallel to the incoming tide, extending to 1 or 2 off-bed stations, and at least two on-bed stations off the transect near along a transect that crosses at the center of the bed, at ½ the distance to the bed along and perpendicular to the tidal current, and at distances of 100 ft and 200 ft outside the bed perimeter along the tidal gradient. Bed-center will be sampled before treatment. Sites will likely be sampled at 1 and 3 days and at 2 and 4 weeks after treatment, with possible additional samples at 8 weeks after treatment, depending on concentrations of previous samples. Some sites will be sampled immediately after (<1 hr) after treatment and some samples will be sampled at 12 hr after treatment (the following low tide). A duplicate sample will be taken at one of the sample sites at each sample date. Pore water sample will be extracted through a small core to a depth of 10 cm.

All water samples will be collected according to USGS “clean hands / dirty hands” Standard Operating Procedures (SOP). Samples will be placed on ice and shipped to the University of Washington, under chain of custody, where they will be analyzed by technicians in the School of Fisheries using an immunoassay (EnviroLogix® Quantiplate Kit) that was validated in 2009 and 2010. Some duplicate samples will be shipped to Pacific Agricultural Laboratories, Portland, Oregon, also under chain of custody, where they will be analyzed to a detection limit of at least 0.01 ppb using standard analytical chemistry techniques.

### Sediment Degradation of Imidacloprid

Imidacloprid concentrations in sediments will be sampled to a depth of 10 cm using a 5.1 cm internal diameter corer. Three cores within 1 m<sup>2</sup> will be collected and homogenized as a single replicate sample. Samples will be collected near sites of pore water sampling, at selected sites where benthic infauna is sampled. Depending on results from the 2010 studies, some samples may be taken at depths > 10 cm. Samples will be placed on ice, shipped to Pacific Agricultural Laboratory, and extracted for imidacloprid analysis within 7 days under chain of custody. Samples will also be analyzed for imidacloprid by using immunoassays at the University of Washington.

Non-target Impacts of Imidacloprid to Crab

Crab and other macrofauna species (WSU): Four study sites will be assessed as indicated in our 2011 EUP applications for imidacloprid, two using 0.5 lbs ai/ac of the 0.5 G formulation and two using the 2 lbs ai/ac of 2 F formulation. Two additional untreated sites will serve as controls. Immediately prior to treatment, juvenile Dungeness crab 0.5 to 2" carapace will be gathered and placed in 1/4" screen mesh cages (10" diameter by 10" height cylinders open on the bottom with wired lid on top. Cages will be set 2" into the sediment to allow crab to bury in mud/sand. For each site there will be 15 cages with 5 crabs per cage. Five additional cages with crabs will be placed on the site after 24 hours, to test for residual effects in the sediment. Crab mobility (tetany) and mortality will be assessed every 24 hours for 4 days. To reduce the chance of cannibalism, crabs will be fed clam meat at each observation. Immediately after and for four days following the treatment, the sites will be surveyed for dead invertebrates and fish, using seven 100 m x 2 m transects per site.

Macrofauna surveys will be conducted at 1 and 24 hr after treatment by counting live, dead, or impaired macrofauna within a 4 m<sup>2</sup> area along transects that cross the bed. Species surveyed will include saddleback gunnels, Pacific staghorn sculpin, bay goby, starry flounder, English sole, and shiner perch, Nereid worms, Crangon shrimp, Scale worms, and Dungeness crab. Any affected crab showing tetany, but still alive, will be collected, taken to the lab and observed for recovery/mortality.

Non-target Impact to Infauna

Non-target impact to infauna on beds treated with imidacloprid will be assessed adjacent to sites of pore water and sediment samples (3 on-bed sites, 2 off-bed sites at each of 100 and 200 ft distances from the bed perimeter along the tidal gradient). Benthic infauna will be sampled pre-treatment, at 3 days post-treatment and at 4 weeks, 8 weeks, and 6 months after treatment interval. Infauna will be sampled using a 10.2 cm corer to a depth of 10 cm, with each core constituting a replicate. Three replicate core samples will be collected per sample site (e.g., 5 sites per sample date). The core will be immediately sieved through 0.5 mm mesh using salt water, then stored in a 10% buffered formalin solution and stained with rose bengal for 1-2 weeks, then re-sieved through 250 um mesh to remove excess detritus and stored in 70% ethanol. Species identification and enumeration will be done by Ruff Wormworks (annelids), UW or Evergreen State College personnel (Crustacea) and mollusks (PSI staff). Species attributes (type and abundance) of key benthic invertebrates, as well as community descriptors (Shannon-Wiener Diversity, Species Evenness, Species Ubiquity, and Species Richness) will be used to compare the benthic infauna among areas of differing levels of imidacloprid and over time. Attributes and descriptors will be further compared using analysis of variance. Community structure will also be examined using classification analyses or ordination methods such as principal components analysis. Key organisms will also be identified and assessed independently as separate measures of impact. According to Washington Administrative Code Sediment Degradation policy, impact will also be assessed according to percent reduction of taxa identified to class.

**3) Objectives**

- Assess the efficacy of a liquid formulation applied at 2 lb a.i./ac and a granular applied at 0.5 lb a.i./ac at the commercial scale.
- Assess application methods of the two formulation / rates, but especially the granular at 0.5 lb a.i./ac.
- Assess non-target impact on crab and other macro-invertebrates, the benthic infauna.
- Measure concentrations of imidacloprid in the water column, in pore-water and in sediments following experimental applications to help evaluate program efficacy and non-target impact.

The experimental program is designed to test the efficacy of the two different formulations of imidacloprid applied at the commercial scale. Previous large scale trials in 2008 demonstrated that the efficacy of the liquid formulation, Nuprid 2F applied at 0.5 lb a.i./ac was not commercially acceptable. An application of the granular material Mallet 0.5G at 0.5 lb a.i./ac to a 9 ac plot in 2009 also showed limited efficacy.



Results of small plot trials of both materials in 2009 and in early spring 2010<sup>2</sup> showed that efficacy of the two different formulation/rates can be inconsistent and likely depends on such factors as type of substrate, bed elevation, and amount of vegetation. These factors vary throughout the bay, requiring treatment of larger acreage to accurately determine best use of the materials.

The trials will also test different application methods. The liquid formulation can easily be applied via the conventional methods for the standard carbaryl-based program: either aerially using helicopters or ground-based sprayer systems. The 0.05% active ingredient in the granular material makes the formulation extremely heavy, complicating application. Application by boat may be simpler than on bare ground yet also improve efficacy, as vegetation would not entirely blanket the ground.

Several studies of non-target impact and fate & transport of imidacloprid in the water column and in sediments are required for the registration of permitting of its use on shellfish beds. While some of these studies have been and continue to be addressed in the laboratory, they also need to be assessed and validated in the field under commercial situations. Data will be gathered this summer to address these studies.

#### **4) Explanation and Justification of Quantity**

These trials will require a maximum of 150 lb a.i. of imidacloprid to be applied to a total of 150 total acres in Willapa Bay or Grays Harbor (180 lb a.i. of Nuprid 2F to 90 ac and 30 lb a.i. of Mallet 0.5G to 60 ac). However, depending on plot availability, the density and distribution of burrowing shrimp in 2011, and the treatment schedule for the conventional carbaryl-based management program for burrowing shrimp, the actual treated acreage could be considerably lower. The requested acreage is required to complete the studies required for imidacloprid registration and permitting in the fourth of a multi-year experimental program. Amounts were derived according to an experimental design that strives for suitable replication but is constrained by limited space, time, and considerations for potential non-target impact. Our most common plot size (5 ac) tend to the low size of most commercial beds ( $\geq 10$  ac) but are still large enough to include some variation in burrowing shrimp density, substrate, vegetation, bed elevation, and drainage pattern that accompany commercial shellfish beds and impact efficacy.

#### **5) Duration**

We request that a federal experimental use permit for imidacloprid on Washington state shellfish grounds be granted for one year.

#### **6) Disposition of unused material**

Almost all imidacloprid will be used during experimental application, as the amount of material applied will be precisely measured and applied using calibrated equipment. Unused material will be stored temporarily in an EPA and OSHA compliant pesticide storage unit located at the Washington State University Research and Extension Unit in Long Beach, WA. Unused material will ultimately be disposed through the Washington Department of Agriculture's Pesticide Disposal Program.

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<sup>2</sup> Conducted under WSEUP no. 09014 and WSEUP no. 10009, which allow yearly total applications of <1ac.

RE: 2011 FEUP Application  
 Steven R. Booth  
 to:  
 Joanne Edwards, John Hebert  
 12/17/2010 02:32 PM  
 Cc:  
 "Kim Patten"  
 Show Details

History: This message has been replied to.

Joanne,

Attached are:

- 1) corrected the 8570-17 forms (I just spaced on the acreage for the Nuprid the other day – forgot it was the 2F) by submitting as “extension” and providing permit number.
- 2) Experimental labels formatted according to previous labels with changes in restrictions related only to a) extended treatment window, b) clarification of area treated in notification signs (include map on signs)
- 3) Attachment 1 with reference to above changes in restrictions from previous years
- 4) Attachment II drops reference to temporary tolerance (that was included in previous applications but I guess it is not relevant to these EUPs.

In a previous email (below) you noted “It (the application) needs to come in through previous channels in order to be processed”. I am not sure what that means – I have previously submitted through you.

I am taking the afternoon off, but will make any other changes or processes if needed on Monday.

Have a good weekend,

Steve

---

**From:** Edwards.Joanne@epamail.epa.gov [mailto:Edwards.Joanne@epamail.epa.gov]  
**Sent:** Thursday, December 16, 2010 12:07 PM  
**To:** Steven R. Booth; Hebert.John@epamail.epa.gov  
**Subject:** RE: 2011 FEUP Application

John needs to weigh in on this, but I see no reason why we can't just "re-extend"..

Then the only thing you would need to do is to resubmit the labels (to like just like what we've already approved) and redo the application form.

I do have a pre-registration package, and did route it for review to EFED. I guess Alan S. will request a meeting early next year. Joanne

-----"Steven R. Booth" <boothswa@comcast.net> wrote: -----



To: Joanne Edwards/DC/USEPA/US@EPA, John Hebert/DC/USEPA/US@EPA  
 From: "Steven R. Booth" <boothswa@comcast.net>  
 Date: 12/16/2010 02:38PM  
 Cc: "Kim Patten" <pattenk@wsu.edu>  
 Subject: RE: 2011 FEUP Application

Thanks Joanne,

I will take care of these items.

However, I can comment on a couple of them now.

I thought this was a "new" application because we have been running on annual EUPs, but I just checked last year's submittal and that was indeed an "extension".

I worked off the labels for our draft proposed labels for final registered product (Protector), but will go back to last year's labels for the EUP.

In the restrictions sections, we did want to expand the treatment window a bit more than last year's permitted window and perhaps decrease the buffers to main channels a bit, but the latter is not critical.

Thank you for your quick response,

Steve

-----Original Message-----

From: Edwards.Joanne@epamail.epa.gov [<mailto:Edwards.Joanne@epamail.epa.gov>]

Sent: Thursday, December 16, 2010 9:35 AM  
 To: Steven R. Booth; Hebert.John@epamail.epa.gov  
 Cc: Steven R. Booth; Kim Patten  
 Subject: Re: 2011 FEUP Application

Hi Steve- I printed out what you submitted and looked over. It needs to come in through normal channels in order to be processed.

I have the following comments:

shouldn't this be an extension (see box 1 on the application form)

What exactly are you doing different? The labels you submitted are missing information (First AID etc.) You need to take the labels we approved last year and resubmit them, Two copies, one which is highlighted in the areas that you have changed. You shouldn't be changing anything in the RESTRICTIONS. And under directions for use, you need the language "To test for efficacy..."

Your application (8570-17) makes no sense. Box number 9 just talks about shipping of material.. This box must have the dates of use. This is an EUP, not a federal registration.

For the liquid product, you have almost doubled the amount of material to be used. But the acreage remains at 90. This makes no sense. The application rate and acrea amount have to add up in the math. This is an

experimental use, not a federal registration, so there are limits to what you can apply

You also need to redo pg 45 of your application, where you talk about PETITION FOR TEMPORARY TOLERANCE. The oysters can't be eaten. This is experimental use only.

Joanne Edwards  
EPA/OPPTS/OPP/RD/IRB  
(703) 305-6736  
edwards.joanne@epa.gov

From: "Steven R. Booth" <boothswa@comcast.net>

To: Joanne Edwards/DC/USEPA/US@EPA

Cc: "Kim Patten" <pattenk@wsu.edu>, "Steven R. Booth" <boothswa@comcast.net>

Date: 12/14/2010 08:57 PM

Subject: 2011 FEUP Application

Hi Joanne,

Attached is the Willapa Grays Harbor Oyster Growers application packet for a Federal Experimental Use Permit to apply imidacloprid on Willapa Bay tidelands in 2011. These include the 8570-17 forms for both Nuprid 2F and Mallet 0.5G, their experimental labels, and Attachments I and II. I have also attached our proposed experimental labels for the flowable and granular products we hope to register soon.

As in previous years, Washington State University (Dr. Kim Patten) is the official submitter.

Please let me know if you need any more information or clarification.

Sincerely,

Steve Booth[attachment "WGHOGA Attachments I & II.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "Mallet 0.5G Exp Label Dec 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "Nuprid 2F Exp Label



Dec 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "WGHOGA  
8570-17 Mallet Dec 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US]  
[attachment "WGHOGA 8570-17 Nuprid Dec 2010.pdf" deleted by Joanne  
Edwards/DC/USEPA/US] [attachment "Proposed Federal 2F Label.pdf" deleted  
by Joanne Edwards/DC/USEPA/US] [attachment "Proposed Federal 0.5G  
Label.pdf" deleted by Joanne Edwards/DC/USEPA/US]

=

# Material to be added to an e-Jacket/Jacket

Reg. No. 86414-ZUP-1

Decision # \_\_\_\_\_

Description:

amended ZUP

1. Placement within the e-Jacket/jacket:

☒ Default: (chronological, top = newest)

☐ File Location: (eg. "before page 45 in .pdf")

\_\_\_\_\_

2. ☒ Send to Data Extraction contractors this material:

☒ Newly stamped accepted label

☐ Notification

☐ New CSF

☐ Other: \_\_\_\_\_

3. Attach this coversheet to the top of the material or jacket. It must be well organized and clipped together, NOT STAPLED. Then give the material with this coversheet to staff in the Information Services Center (Room S-4900).

Reviewer: Joanne Edwards

Division: RD

Phone: 305-6736

Date: 3/12/16





UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

Dr. Kim Patten  
Washington State University  
Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach, WA 98631

MAR 17 2010

Dear Dr. Patten;

Subject: Amended Experimental Use Permit  
Experimental Use Permit No. 86414-EUP-1  
Effective Dates: May 1, 2010 to October 31, 2010  
Quantity Authorized: 100 pounds of active ingredient  
Your Application Dated February 9, 2010

There is no objection to the change in language on the label for the subject experimental use permit from:

"Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between June 1 and October 31." to read: "All applications must occur between May 1 and October 31."

A stamped copy of the label is enclosed for your records. This labeling must be used for all shipments of this product under the subject EUP.

If you have any questions in reference to this permit, contact me at (703) 305-6736.  
Sincerely,

Sincerely,

Joanne S. Edwards  
Insecticide-Rodenticide Branch  
Registration Division 7505P  
Office of Pesticide Programs

cc: EPA Region: 10

# NUPRID 2F

## FOR EXPERIMENTAL USE ONLY

Experimental Use Permit Number:

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

---

**Permittee:**

Kim Patten, Extension Specialist, Professor  
Washington State University Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach WA 98631

---

**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine . . . . .21.4%

OTHER INGREDIENTS: . . . . .78.6%

TOTAL: . . . . .100.0%

Contains 2 pounds of imidacloprid per gallon.

## KEEP OUT OF REACH OF CHILDREN

### CAUTION – CAUCION

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No. 86414-EUP-1

ACCEPTED

For shipment and use of product for experimental  
purposes under the provision of the Federal Insecticide,  
Fungicide, and Rodenticide Act, subject to attached  
comments.

Permit No. 86414-EUP-1

Issued on January 6, 2010

and amended on March 17, 2010



FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
NOTE TO PHYSICIAN	
No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS  
HAZARDS TO HUMANS AND DOMESTIC ANIMALS  
CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
- Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
- Shoes plus socks
- Protective eyewear when working in a non-ventilated space

Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Users must:**

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.

**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 2.0 lb a.i./ac using the following properly calibrated application equipment:

- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~20 gpa.
- SpikeWheel™ spoke wheel subsurface injectors operated from a floating platform at ~20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500ft.
- For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.
- All applications must occur between May 1 and October 31.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within a one-and-a-half (1 1/2) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (1/4) of an inch high. Signs shall also state "Do Not Fish, Crab, or Clam". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

**SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.



### **Restrictions During Temperature Inversions**

Because the potential for spray drift is high during temperature inversions, do NOT make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing, which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

### **Importance of Droplet Size**

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

### **Mixing and Loading Requirements**

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

### **STORAGE AND DISPOSAL**

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Refer to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk through spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak incidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.





Re: questions  
Steven R. Booth to: Joanne Edwards

03/17/2010 12:03 PM

Joanne,

We want to move treatments up to May 1 as developing eelgrass stands (an estuarine ribbonny plant) get really dense by early June, blocking efficacy of the chemical.

I am not sure why I had that dangling reference to aerial and ground-based application hanging there. I try to go over these changes very carefully, but somehow did not get it right.

That particular restriction should simply read: "All applications must occur between May 1 and October 31." for both formulations.

I hope you had a good break. You had lots of snow, right?

----- Original Message -----

From: <Edwards.Joanne@epamail.epa.gov>  
To: "Steven R. Booth" <boothswa@comcast.net>  
Sent: Tuesday, March 16, 2010 12:14 PM  
Subject: Re: questions

> Steve- I.m back from skiing and trying to do catch yup. I prepared  
> letters for you, but need to know what's with the statement  
>  
> Aerial applications (not ground-based topical applications and  
> subsurface injection), all applications must occur between June 1 and  
> October 31  
>  
>  
> I can understand "Aerial applications must occur between June 1 and  
> October 31." (then you need to say when the other types of  
> application can occur)  
>  
> or "All applications must occur between June 1 and October 31"  
>  
> explain please  
>  
>  
>  
> Joanne Edwards  
>  
>  
>  
> EPA/OPPTS/OPP/RD/IRB  
> (703) 305-6736  
> edwards.joanne@epa.gov  
>  
>  
> |----->  
> | From: |

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> |----->  
> | "Steven R. Booth" <boothswa@comcast.net>

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> | To: |

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> | Joanne Edwards/DC/USEPA/US@EPA

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> | Date: |

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> | 02/17/2010 03:53 PM

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> | Subject: |

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> | Re: questions

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> Thanks Joanne,

> Previous language on the granular label read (in RESTRICTIONS):

> For aerial and ground-based topical applications and ground-based

> subsurface injection, all applications must be on beds exposed at low



> tide. Subsurface injections from a floating platform must be applied to  
> beds under water.  
>  
> Aerial applications (not ground-based topical applications and  
> subsurface injection), all applications must occur between July 1 and  
> October 31.  
>  
> New (requested) language reads:  
>  
> Aerial applications must be on beds exposed at low tide. Applications  
> from a floating platform or boat may be applied to beds under water.  
>  
>  
> Aerial applications (not ground-based topical applications and  
> subsurface injection), all applications must occur between May 1 and  
> October 31.  
>  
>  
> New language on the flowable label is just the change in application  
> date from June 1 to May 1.  
>  
> We will give you a new study plan for your information once we have it  
> detailed. Total acreage will not change.  
>  
> Steve  
>  
> ----- Original Message -----  
> From: <Edwards.Joanne@epamail.epa.gov>  
> To: "Steven R. Booth" <boothswa@comcast.net>  
> Sent: Wednesday, February 17, 2010 11:44 AM  
> Subject: Re: questions  
>  
>> Steve- I just made copies and I'm having them put into the system.  
>> can you tell me exactly what language has changed on the granular  
> label?  
>>  
>> I don't think you need to submit an updated study plan since the  
> acreage  
>> is the same, unless you want to.  
>>  
>> I'll be in Tahoe week starting 3/6 for week of skiing.  
>>  
>> I'll get back to you once I get the applications put in the system,  
> and  
>> look them over,  
>>  
>>  
>>  
>> Joanne Edwards  
>> EPA/OPPTS/OPP/RD/IRB  
>> (703) 305-6736  
>> edwards.joanne@epa.gov  
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>  
>> From: "Steven R. Booth" <boothswa@comcast.net>  
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>> To: Joanne Edwards/DC/USEPA/US@EPA

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>> Date: 02/09/2010 12:15 PM  
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>>  
>  
>> Subject: Re: questions  
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>>  
>> Hi Joanne,  
>>  
>> I have attached the letters and altered labels. I made the letters  
> out  
>> to  
>> Merideth Laws and John Hebert. Should I include you or make them out  
> to  
>>  
>> only you?  
>>  
>> We also wish to alter our experimental plan somewhat as it is  
> presented  
>> in  
>> attachment 2 or our application for the EUP last year. In that plan,  
>> our  
>> experimental plot sizes ranged from 2.5 to 10 acres with perhaps a 20  
> ac  
>>  
>> application. We would like to treat some smaller plots, ranging from  
>> 0.5 to  
>> 1 ac, as we are still trying to sort out which of the materials (the  
> 2F  
>> or  
>> the 0.5G) works best on which sorts of substrates and in different  
>> conditions. Total acreage would stay the same. Should we submit an  
>> updated  
>> study plan?  
>>  
>> Thanks, as always, for you help.  
>>  
>> BTW, we will be holding a workshop of growers and local agency folks,  
> on  
>>  
>> March 11 (maybe 12) to present last year's data and discuss future  
>> plans, if  
>> you or somebody from your shop would want to attend.  
>>  
>> Steve  
>> ----- Original Message -----  
>> From: <Edwards.Joanne@epamail.epa.gov>  
>> To: "Steven R. Booth" <boothswa@comcast.net>  
>> Sent: Tuesday, January 19, 2010 7:33 AM  
>> Subject: Re: questions  
>>  
>>



>>> Hi Steve- I think we could treat as amendments. Submit one letter  
>>> with rationale for earlier start date.  
>>>  
>>> Submit another letter (and label) with the revised directions for use  
>>> and label. You can do it via e-submission.  
>>>  
>>> Joanne Edwards  
>>> EPA/OPPTS/OPP/RD/IRB  
>>> (703) 305-6736  
>>> edwards.joanne@epa.gov  
>>>  
>>>  
>>> From: "Steven R. Booth" <boothswa@comcast.net>  
>>>  
>>> To: Joanne Edwards/DC/USEPA/US@EPA  
>>>  
>>> Date: 01/15/2010 02:19 PM  
>>>  
>>> Subject: questions  
>>>  
>>>  
>>>  
>>>  
>>>  
>>> Hi Joanne,  
>>>  
>>> We have received our FEUPs for applications of the two formulations  
> of  
>>> imidacloprid on SW Washington shellfish beds.  
>>>  
>>> Thanks for all your help.  
>>>  
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>>> small plot (<0.1 ac) studies conducted under the State EUP showed  
> that  
>>> applications in May were much more effective than later applications,  
>> as  
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>>>  
>>> Also, small plot trials indicated that the granular formulation was  
>> also  
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>>> anyway.  
>>>  
>>> Can you help me with these questions? Will such changes require a  
>> whole  
>>> new application or can we just amend the current EUP?  
>>>  
>>> Thanks,  
>>>  
>>> Steve Booth  
>>>  
>>>  
>>>  
>> [attachment "MALLET EXP LABEL 86414-2 Feb 2010.pdf" deleted by Joanne  
>> Edwards/DC/USEPA/US] [attachment "EXP LABEL Use Amendment Request  
>> 86414-2.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "EXP  
>> LABEL Amendment earlier app date request 86414-1 & 2.pdf" deleted by  
>> Joanne Edwards/DC/USEPA/US] [attachment "NUPRID EXP LABEL 86414-EUP-1  
>> Feb 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US]  
>>  
>>  
>>  
>  
>  
>



**NUPRID 2F**

**FOR EXPERIMENTAL USE ONLY**

Experimental Use Permit Number:

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

---

**Permittee:**

Kim Patten, Extension Specialist, Professor  
Washington State University Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach WA 98631

---

**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine . . . . . 21.4%

**OTHER INGREDIENTS:** . . . . . 78.6%

**TOTAL:** . . . . . 100.0%

Contains 2 pounds of imidacloprid per gallon.

**KEEP OUT OF REACH OF CHILDREN**

**CAUTION – CAUCION**

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No. 86414-EUP-1

FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
NOTE TO PHYSICIAN	
No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS  
HAZARDS TO HUMANS AND DOMESTIC ANIMALS  
CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
  - Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
  - Shoes plus socks
  - Protective eyewear when working in a non-ventilated space
- Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Users must:**

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.

**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 2.0 lb a.i./ac using the following properly calibrated application equipment:

- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~ 20 gpa.
- SpikeWheel™ spoke wheel subsurface injectors operated from a floating platform at ~20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500ft.
- For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.
- Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between May 1 and October 31.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within a one-and-a-half (1 1/2) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (1/4) of an inch high. Signs shall also state "Do Not Fish, Crab, or Clam". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

**SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.



### **Restrictions During Temperature Inversions**

Because the potential for spray drift is high during temperature inversions, do NOT make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing, which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

### **Importance of Droplet Size**

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

### **Mixing and Loading Requirements**

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

### **STORAGE AND DISPOSAL**

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Refer to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk through spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak incidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.

Re: questions  
Steven R. Booth  
to:  
Joanne Edwards  
02/17/2010 03:53 PM  
Show Details

History: This message has been replied to.  
Thanks Joanne,

Previous language on the granular label read (in RESTRICTIONS):

For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.

Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between July 1 and October 31.

New (requested) language reads:

Aerial applications must be on beds exposed at low tide. Applications from a floating platform or boat may be applied to beds under water.

Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between May 1 and October 31.

New language on the flowable label is just the change in application date from June 1 to May 1.

We will give you a new study plan for your information once we have it detailed. Total acreage will not change.

Steve

----- Original Message -----



From: <[Edwards.Joanne@epamail.epa.gov](mailto:Edwards.Joanne@epamail.epa.gov)>  
 To: "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>  
 Sent: Wednesday, February 17, 2010 11:44 AM  
 Subject: Re: questions

> Steve- I just made copies and I'm having them put into the system.  
 > can you tell me exactly what language has changed on the granular label?  
 >  
 > I don't think you need to submit an updated study plan since the acreage  
 > is the same, unless you want to.  
 >  
 > I'll be in Tahoe week starting 3/6 for week of skiing.  
 >  
 > I'll get back to you once I get the applications put in the system, and  
 > look them over,  
 >  
 >  
 >

> Joanne Edwards  
 > EPA/OPPTS/OPP/RD/IRB  
 > (703) 305-6736  
 > [edwards.joanne@epa.gov](mailto:edwards.joanne@epa.gov)  
 >  
 >  
 >

> From: "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>  
 >  
 > To: Joanne [Edwards/DC/USEPA/US@EPA](mailto:Edwards/DC/USEPA/US@EPA)  
 >  
 > Date: 02/09/2010 12:15 PM  
 >  
 > Subject: Re: questions  
 >  
 >  
 >  
 >  
 >  
 >

> Hi Joanne,  
 >

> I have attached the letters and altered labels. I made the letters out  
 > to  
 > Merideth Laws and John Hebert. Should I include you or make them out to  
 >  
 > only you?  
 >  
 > We also wish to alter our experimental plan somewhat as it is presented  
 > in  
 > attachment 2 or our application for the EUP last year. In that plan,  
 > our  
 > experimental plot sizes ranged from 2.5 to 10 acres with perhaps a 20 ac  
 >  
 > application. We would like to treat some smaller plots, ranging from  
 > 0.5 to  
 > 1 ac, as we are still trying to sort out which of the materials (the 2F  
 > or  
 > the 0.5G) works best on which sorts of substrates and in different  
 > conditions. Total acreage would stay the same. Should we submit an  
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 >

> Steve

> ----- Original Message -----

> From: <[Edwards.Joanne@epamail.epa.gov](mailto:Edwards.Joanne@epamail.epa.gov)>  
 > To: "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>  
 > Sent: Tuesday, January 19, 2010 7:33 AM  
 > Subject: Re: questions  
 >  
 >

>> Hi Steve- I think we could treat as amendments. Submit one letter  
 >> with rationale for earlier start date.  
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>> Submit another letter (and label) with the revised directions for use  
 >> and label. You can do it via e-submission.  
 >>

>> Joanne Edwards  
 >> EPA/OPPTS/OPP/RD/IRB  
 >> (703) 305-6736  
 >> [edwards.joanne@epa.gov](mailto:edwards.joanne@epa.gov)  
 >>  
 >>

>> From: "Steven R. Booth" <[boothswa@comcast.net](mailto:boothswa@comcast.net)>  
 >>  
 >> To: Joanne [Edwards/DC/USEPA/US@EPA](mailto:Edwards/DC/USEPA/US@EPA)  
 >>  
 >> Date: 01/15/2010 02:19 PM  
 >>  
 >> Subject: questions  
 >>  
 >>  
 >>  
 >>

>> Hi Joanne,  
 >>

>> We have received our FEUPs for applications of the two formulations of  
 >> imidacloprid on SW Washington shellfish beds.  
 >>

>> Thanks for all your help.  
 >>

>> However, as I indicated in an earlier email, results from last years  
 >> small plot (<0.1 ac) studies conducted under the State EUP showed that  
 >> applications in May were much more effective than later applications,  
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>> stands of eelgrass increase greatly during June and July and prevent  
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>> the material getting to the bed. Earlier applications would also  
 >> hypothetical reduce off-site movement due to the same reasoning.  
 >>

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 >> treatment from "between May 1 to October 31" rather than " between  
 > June



>> 1 to October 31"?

>>

>> Also, small plot trials indicated that the granular formulation was

> also

>> effective when applied from a boat when the beds were flooded, as the

>> heavy material sank straight down. The material is difficult to apply

>> by hand on the low tides, as it is so heavy.

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>> Would it be possible to change the label for the Mallet (granular) to

>> allow such applications? The current relevant restriction on the

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>> tide. Subsurface injections from a floating platform must be applied

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>> beds under water". We have pretty much given up on the subsurface

>> injections because they did not work and the "floating platforms" were

>> too hard to maneuver. The granular won't go through the injectors

>> anyway.

>>

>> Can you help me with these questions? Will such changes require a

> whole

>> new application or can we just amend the current EUP?

>>

>> Thanks,

>>

>> Steve Booth

>>

>>

>>

>>

> [attachment "MALLET EXP LABEL 86414-2 Feb 2010.pdf" deleted by Joanne

> Edwards/DC/USEPA/US] [attachment "EXP LABEL Use Amendment Request

> 86414-2.pdf" deleted by Joanne Edwards/DC/USEPA/US] [attachment "EXP

> LABEL Amendment earlier app date request 86414-1 & 2.pdf" deleted by

> Joanne Edwards/DC/USEPA/US] [attachment "NUPRID EXP LABEL 86414-EUP-1

> Feb 2010.pdf" deleted by Joanne Edwards/DC/USEPA/US]

>

>

>

# Willapa-Grays Harbor Oyster Growers Association

P.O. Box 3 Ocean Park, WA 98640

**From:**

Steven R. Booth, Ph.D.  
IPM Coordinator, WGHOGA  
Senior Scientist, Pacific Shellfish Institute  
2711 44<sup>th</sup> Ave. N.W.  
Olympia, WA 98502  
360-867-4163  
[boothswa@comcast.net](mailto:boothswa@comcast.net)  
[booths@pacshell.org](mailto:booths@pacshell.org)

Tim Morris  
President, WGHOGA  
P.O. Box 3  
Ocean Park, WA 98640

Dr. Kim Patten  
Extension Specialist, Professor  
Washington State University  
Long Beach Research Unit  
2907 Pioneer Road  
Long Beach WA 98631  
360-642-2031  
[pattenk@wsu.edu](mailto:pattenk@wsu.edu)

**To:**

Meredith Laws, Branch Chief  
Insecticide-Rodenticide Branch  
Registration Division  
USEPA

John Hebert, PM 7 USEPA  
Insecticide-Rodenticide Branch  
Registration Division  
Rom S-4900  
One Potomac Yard  
2777 South Crystal Drive  
Arlington, VA 22202-4501

February 9, 2010

**RE:** Amendment of Experimental Labels 86414-EUP-1 and 86414-EUP-2 to expand seasonal application to May 1 – Oct 31.

Dear Drs. Laws and Hebert:

Thank you for granting our applications for Federal Experimental Use Permits to treat acreage of Willapa Bay, Washington shellfish grounds with two different formulations of imidacloprid.

However, we wish to amend the experimental labels slightly to allow earlier applications of both formulations. The current experimental label for Nuprid 2F restricts its use to between June 1 and October 31. The current experimental label for Mallet 0.5G restricts its use to between July 1 and October 31. We wish to expand the application interval for both materials to between May 1 and October 31. Experimental applications on small plots during May were more effective against burrowing shrimp than July applications, as patches of eelgrass are still relatively sparse. Both Japanese and native eelgrass become quite dense during late June and July, obstructing the ability of the pesticides to contact the bed surface.

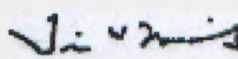
I have modified the experimental labels for both materials to reflect this changes and have also added the experimental label numbers, 86414-EUP-1 and 86414-EUP-2.

Hopefully these changes are acceptable and can be handled as amendments to the labels.

Sincerely,



Steven R. Booth



Tim Morris



Kim Patten





UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES  
AND TOXIC SUBSTANCES

Dr. Kim Patten  
Washington State University  
Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach, WA 98631

JAN 06 2010

Dear Dr. Patten;

Subject: Experimental Use Permit  
Active Ingredient: Imidacloprid  
Experimental Use Permit No. 86414-EUP-1  
Effective Dates: May 1, 2010 to October 31, 2010  
Quantity Authorized: 100 pounds of active ingredient

On the basis of information furnished by the applicant, an Experimental Use Permit (EUP) under section 5 of the Federal Insecticide, Fungicide, and Rodenticide Act, as amended (86 Stat. 983), is hereby issued for the active ingredient imidacloprid (NUPRID 2F, EPA Reg. No. 81959-22). This permit authorizes use of NUPRID 2F to investigate the efficacy of the product as burrowing shrimp control agent in oyster and manila clam beds in Willapa Bay and Grays Harbor, Washington. Shipment and/or use under this permit is subject to the provisions of 40 CFR 172.

PRIOR TO SHIPMENT AND/OR USE OF THIS MATERIAL, YOU MUST CONSULT WITH THE STATE PESTICIDE REGULATORY OFFICIALS OF THE STATES IN WHICH YOUR EXPERIMENTAL PROGRAM WILL BE CONDUCTED AND OBTAIN A STATE PERMIT OR LICENSE IF SUCH IS REQUIRED. ISSUANCE OF THIS FEDERAL PERMIT DOES NOT NEGATE THE NEED FOR PERMISSION FROM INDIVIDUAL STATES. FAILURE TO DO SO MAY RESULT IN REVOCATION OR MODIFICATION OF THIS EXPERIMENTAL USE PERMIT. A DIRECTORY OF STATE PESTICIDE CONTROL OFFICIALS CAN BE FOUND UNDER THE AAPCO WEBSITE: <http://www.aapco.ceris.purdue.edu/htm/control.htm>.

Based upon the experimental program submitted, this product may be shipped for use under this permit to Washington, to treat up to 80 acres of oyster/manila clam beds located in Willapa Bay and Grays Harbor, Washington.

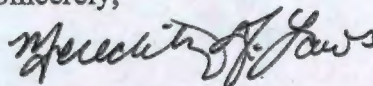
The labeling submitted in connection with the application for this EUP is acceptable, subject to the following comments:

- a. Add the EPA Experimental Use Permit Number, 86414-EUP-1.

A stamped copy of the label is enclosed for your records. This labeling must be used for all shipments of this product under the subject EUP.

If you have any questions in reference to this permit, contact Joanne Edwards at (703) 305-6736 or electronically at [edwards.joanne@epa.gov](mailto:edwards.joanne@epa.gov).

Sincerely,

A handwritten signature in black ink, appearing to read "Meredith F. Laws", written in a cursive style.

Meredith F. Laws, Chief  
Insecticide-Rodenticide Branch  
Registration Division 7505P  
Office of Pesticide Programs

Enclosure

cc: EPA Region 10



# NUPRID 2F

## FOR EXPERIMENTAL USE ONLY

Experimental Use Permit Number:

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

---

**Permittee:**

Kim Patten, Extension Specialist, Professor  
Washington State University Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach WA 98631

---

**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine .....21.4%

OTHER INGREDIENTS: .....78.6%

TOTAL: .....100.0%

Contains 2 pounds of imidacloprid per gallon.

## KEEP OUT OF REACH OF CHILDREN

### CAUTION – CAUCION

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No.

ACCEPTED

For shipment and use of product for experimental  
purposes under the provision of the Federal Insecticide,  
Fungicide, and Rodenticide Act, subject to attached  
comments.

Permit No.

86414 EUP-1

Issued on

January 6, 2010

FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
NOTE TO PHYSICIAN	
No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS  
HAZARDS TO HUMANS AND DOMESTIC ANIMALS  
CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
  - Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
  - Shoes plus socks
  - Protective eyewear when working in a non-ventilated space
- Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Users must:**

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.  
**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 2.0 lb a.i./ac using the following properly calibrated application equipment:

- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~ 20 gpa.
- SpikeWheel™ spoke wheel subsurface injectors operated from a floating platform at ~20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500ft.
- For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.
- Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between June 1 and October 31.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within a one-and-a-half (1 1/2) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (1/4) of an inch high. Signs shall also state "Do Not Fish, Crab, or Clam". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

**SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.



### Restrictions During Temperature Inversions

Because the potential for spray drift is high during temperature inversions, do NOT make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing, which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

### Importance of Droplet Size

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

### Mixing and Loading Requirements

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

### STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Referr to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk thorough spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak insidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.

# Material to be added to an e-Jacket/Jacket

Reg. No. 86414-EUP-1

Decision # \_\_\_\_\_

Description:

Oyster EUP

1. Placement within the e-Jacket/jacket:

☒ Default: (chronological, top = newest)

☐ File Location: (eg. "before page 45 in .pdf")  
\_\_\_\_\_

2. ☒ Send to Data Extraction contractors this material:

☒ Newly stamped accepted label

☐ Notification

☐ New CSF

☐ Other: \_\_\_\_\_

3. Attach this coversheet to the top of the material or jacket. It must be well organized and clipped together, NOT STAPLED. Then give the material with this coversheet to staff in the Information Services Center (Room S-4900).

Reviewer: Joanne Edwards

Division: RD

Phone: 305-6736

Date: 11/6/10



## INSTRUCTIONS

Refer to 40 CFR 172 for regulations regarding experimental use permits. These regulations were published in the FEDERAL REGISTER on April 30, 1975 (40 FR 18780). Complete all (and only) numbered items on the application form. If an EPA Company Number (Item 2) has not previously been assigned, indicate "None," and a number will be assigned on your acknowledgment copy of the form. Third party applicants (those who will be testing another firm's registered product) need not complete Item 13. On the acknowledgment copy of this form, you will be assigned a File Number or Symbol for identification of this application. An expected completion date and the name of your EPA Contact will be entered. You may call your EPA Contact if you have not received your permit or a letter of explanation by the date indicated.

### Experimental Use Permit Data Submission

The following information must be submitted in triplicate and in detail (bound in removable sections A through G with margin tabs) for all new chemicals and many new products. For some new formulations, the information requested in Items C, D, E, and F may be included by reference to other formulations if adequate extrapolation may be made. Where the applicant requests permission to test a registered product, the information requested in Items B, E, F, and G below, along with the EPA Registration Number of the product, will usually suffice. Refer to 40 CFR 158.640 [53 FR 15993, May 4, 1988] for further information.

- A. A data sheet giving the chemical and physical properties of the chemical. A complete statement of the names and percentages by weight of each Active and Inert ingredient in the formulation to be shipped. This information will be handled as confidential material.
- B. One copy of the proposed label including directions for use necessary for evaluation of the product. Refer to 40 CFR 172.6 for minimum labeling requirements. In certain circumstances the experimental program or other supplemental labeling may be permissible in lieu of full labeling. In such cases, submit a full explanation as to how the labeling will be affixed to or accompany the container.
- C. Toxicity data or reference to available data on the toxicity of the pesticide including, where pertinent, data on the toxicity to fish and wildlife. Include a summary of this information. LD<sub>50</sub> values and results of eye irritation studies on the formulated product must be included.
- D. Residue data, where pertinent, on (a) food or feed commodities; (b) nonfood crops such as tobacco; and (c) foliage or other sites which may relate to worker hazard or adverse effects on the environment. Include a description of the analytical method(s) used and a summary of the data.
- E. Effectiveness data [required only if specified in Regulations 40 CFR 158.640, 53 FR 15993, May 4, 1988 and Registration Guidelines 40 CFR 158.202(i), 53 FR 15993, May 4, 1988].
- F. If the pesticide is to be tested in a manner involving food or feed, and an adequate tolerance is not established to cover the use, file a petition for a temporary tolerance with this Agency and forward three copies with this application. If appropriate tolerances are established already, cite applicable Regulation in Title 40 of the Code of Federal Regulations.
- G. Proposed Experimental Program:
  - (1) Give the qualifications and the names, addresses, and telephone numbers of the individuals (participants) who will supervise the experimental work.
  - (2) Name the States in which the pesticide will be used and the acreage to be treated in each State. Where "acreage" does not apply, give extent of testing per State in more appropriate terminology. Indicate separately any other State(s) to which the pesticide may be shipped for further distribution.
  - (3) Give the details of the proposed program including the types of target pests or organisms, the crops, animals, surfaces, materials, buildings, or sites of application to be treated and the major geographical areas where the material is to be used. For seasonal pests or crops, indicate the desired month for pesticide application to begin. Specify the use pattern, intended plot sizes, number of plots, number of replicates, dosage rates, methods of application, season of use (spring, summer, fall) and timing of application (preplant, postemergence, multiple (indicate pattern and number), etc.).
  - (4) List the objectives of the proposed program including, e.g., what type(s) of data will be collected during the testing period (performance, yield, phytotoxicity, environmental residue, etc.). Indicate your long-range testing plans, including how many years you expect to conduct experimental testing in support of registration of this use. This information will be helpful in evaluating the currently proposed program.
  - (5) Submit an explanation to justify the quantity of the material requested, including various parameters used to determine the quantity. Quantities authorized will be based on the program submitted and consideration of the types and amount of data required to support registration.
  - (6) Propose a suitable duration for the permit commensurate with the program. Any request for a period greater than 1 year must be adequately justified.
  - (7) State the method of disposition of any unused material left at the conclusion of the testing program.

### Paperwork Reduction Act Notice

The public reporting burden for this collection of information is estimated to average three quarters of an hour including time for reviewing instructions, gathering existing product sources and addresses, shippers to be used and addresses, and completing this instrument. Send comments regarding this estimate or any other aspect of this process, including suggestions for reducing the burden to: Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460; Office of Management and Budget, Paperwork Reduction Project (2070-0040), Washington, DC 20503.

NOTE: Applicant may retain last copy  
(04-14-93)



Form Approved, OMB No. 2070-0040.

OPP Identifier Number



United States  
**ENVIRONMENTAL PROTECTION AGENCY**  
 Washington, DC 20460

Office of Pesticides Programs (7505C)

**Application for Experimental Use Permit to Ship and  
 Use a Pesticide for Experimental Purposes Only**

**1. Type of Application**

New



Amendment (See No. 2)



Extension (Give Permit Number below)

Permit Number

**2. Briefly explain (attach a separate sheet if necessary)**

This EUP is to be used to investigate the efficacy and nont-target effects of imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor, Washington.

**3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be Issued (Include Zip Code) (Type or Print)**

Kim Patten, Extension Specialist, Professor  
 Washington State University Long Beach Research and Unit  
 2907 Pioneer Road  
 Long Beach WA 98631

**4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (Include Zip Code) (Type or Print)**

Nufarm Americas Inc.  
 150 Harvester Dr., Suite 200  
 Burr Ridge, IL 60527

EPA Company Number 81959-22

**5. Name of Product**

Name of registered product: Nuprid 2F

**6. Is Product Registered with EPA?**

No



Yes (Give Registration Number or File Symbol below)

Registration Number EPA Reg. No. 228-484

File Symbol

**7. Total Quantity of Product Proposed for Shipment/Use**

Pounds of formulated product 417

Pounds of active ingredient 100

**8. Acreage or Area to be Treated**

maximum 80(30 ac@2 lb a.i./ac, 30  
 ac@1 lb a.i./ac, 20 ac@0.5 lb a.i./ac)

**9. Proposed Period of Shipment/Use**

May 2010 -- October 2010

**10. Places from which Shipped**

Nufarm Inland Empire Dist  
 1211E St Helens ST STE B, Pasco, WA 99301

**11. Crop/Site to be Treated**

Oysters and Manila Clams (Tapes philippinarum)  
 Willapa Bay and Grays Harbor, Washington

**12. Specify the name and number of the contact person most familiar with this application.**

Kim Patten 360-642-2031  
 Steven R. Booth 360-867-4163

**13. Signature of Applicant or Authorized Firm Representative**
**14. Title**

WBGHOGA IPM Coordinator

**15. Date Signed**

12/10/2009

**Certification**

This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.

I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.

**Below for EPA Use Only**

In any correspondence on this application, refer to this number

Received by:  
 EPA-OPP Registration Division,  
 Washington, DC 20460

Normal review time indicates that processing of this application should be completed by (date)

Name of EPA Contact Person

Telephone Number



## INSTRUCTIONS

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NOTE: Applicant may retain last copy  
(04-14-93)



Re: oyster EUPS  
Steven R. Booth to: Joanne Edwards

12/10/2009 02:15 PM

Joanne,  
Part of the confusion of the request is that I broke up the acreage for each product / formulation into different rates -- for comparison purposes. See page 33 of Attachments 1 & 2 - but I have also attached a spreadsheet that shows the breakdown and calculations.

So the 80-17 for the liquid Nuprid was almost correct -- actually lbs of formulated product is 417 lbs rather than 415 if you carry out the decimal places of weight of water (8.34543 lbs per gal); total lb a.i. is 100.

The 80-17 for the granular Mallet was actually too low for the total a.i.. It should be 40 lbs a.i. rather than 30 because different acreages are being treated with different rates. Again see attached spreadsheet. Amazingly, the weight of formulated product is indeed 5000 lbs. The amount is so mind-boggling high that I have to refigure every time because it just seems too high. But the Mallot .5G product has a percentage (underlined) a.i. of .5....leaving 99.5% of the weight accounted for by the inerts.

I have attached corrected forms for both products.

If all this makes the fomrs too confusing, I could simplify.

Also, will there any chance to modify the exp design at all this spring? We may want to fly on some of the granular, just because it is so heavy. Probably limited acreage.

Thanks again for all your help on this.

Steve Booth  
360-867-4163

----- Original Message -----

From: <Edwards.Joanne@epamail.epa.gov>  
To: "Steven R. Booth" <boothswa@comcast.net>  
Sent: Wednesday, December 09, 2009 11:15 AM  
Subject: oyster EUPS

>  
> (See attached file: EFED REVIEW for Oyster EUPS.doc)  
>  
> Steve- here's EFED review.  
>  
> I haven't seen FR Notice, although I know it was published. We can't  
> issue until after the 30 days have expired.  
>  
> I was drafting letter, and darn, your application form is still  
> incorrect, If the request s to treat up to 80 acres, then how come you  
> want 100 pounds of active ingredient (The product contains 21.4%  
> imididacloprid).  
>  
>  
> Same errors on the granular (you need 300 pounds ai for 30 acres???)  
>



>  
> Please redo these forms again, and resubmit electronically. We don't  
> want to authorize use of more product than is needed for the testing  
> program!

>  
> Joanne Edwards  
> EPA/OPPTS/OPP/RD/IRB  
> (703) 305-6736  
> edwards.joanne@epa.gov



WGHOGA 8570-17 Mallet Dec 2009.pdf WGH0HA 8570-17 Nuprid Dec 2009.pdf FEUP imidacloprid rate calcs.xlsx

formulation	ac	lb a.i. / ac	lb a.i.	gal /ac	gal	lb / gal	lb form
Nuprid 2F	20	0.5	10	0.25	5	8.345264	41.72632
	30	1	30	0.5	15	8.345264	125.17896
	30	2	60	1	30	8.345264	250.35792
	TOTAL:		100			TOTAL:	417.2632
					50	8.345264	417.2632

formulation	ac	lb a.i. / ac	% a.i.	total a.i.	form/ac	total form
Mallet 0.5G	1	1	50	1.00	2	2 example
	1	1	5	1.00	20	20 example
	1	1	0.5	1.00	200	200 example
	1	0.5	0.5	2.00	100	100 example
	0.5	0.5	0.5	1.00	50	50 example

10	0.5	0.5	20.00	10	1000 FEUP
20	1	0.5	20.00	40	4000 FEUP
TOTAL:			40	TOTAL:	5000 FEUP





**oyster EUPS**  
**Joanne Edwards** to: Steven R. Booth

12/09/2009 02:15 PM



EFED REVIEW for Oyster EUPS.doc

Steve- here's EFED review.

I haven't seen FR Notice, although I know it was published. We can't issue until after the 30 days have expired.

I was drafting letter, and darn, your application form is still incorrect, If the request s to treat up to 80 acres, then how come you want 100 pounds of active ingredient (The product contains 21.4% imididacloprid).

Same errors on the granular (you need 300 pounds ai for 30 acres???)

Please redo these forms again, and resubmit electronically. We don't want to authorize use of more product than is needed for the testing program!

Joanne Edwards  
EPA/OPPTS/OPP/RD/IRB  
(703) 305-6736  
edwards.joanne@epa.gov

and page number).

- ii. Follow directions. The Agency may ask you to respond to specific questions or organize comments by referencing a Code of Federal Regulations (CFR) part or section number.
- iii. Explain why you agree or disagree; suggest alternatives and substitute language for your requested changes.
- iv. Describe any assumptions and provide any technical information and/or data that you used.
- v. If you estimate potential costs or burdens, explain how you arrived at your estimate in sufficient detail to allow for it to be reproduced.
- vi. Provide specific examples to illustrate your concerns and suggest alternatives.
- vii. Explain your views as clearly as possible, avoiding the use of profanity or personal threats.
- viii. Make sure to submit your comments by the comment period deadline identified.

3. Environmental justice. EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of any group, including minority and/or low income populations, in the development, implementation, and enforcement of environmental laws, regulations, and policies. To help address potential environmental justice issues, the Agency seeks information on any groups or segments of the population who, as a result of their location, cultural practices, or other factors, may have atypical or disproportionately high and adverse human health impacts or environmental effects from exposure to the pesticide(s) discussed in this document, compared to the general population.

## II. What Action is the Agency Taking?

Under section 5 of FIFRA, 7 U.S.C. 136c, EPA can allow manufacturers to field test pesticides under development. Manufacturers are required to obtain EUPs before testing new pesticides or new uses of pesticides if they conduct experimental field tests on 10 acres or more of land or one acre or more of water.

Pursuant to 40 CFR 172.11(a), the Agency has determined that the following EUP applications may be of regional and national significance, and therefore is seeking public comment on the EUP applications:

Submitter: Washington State University Long Beach Research Unit, (86414EUPe and 86414EUPR).

Pesticide Chemical: Imidacloprid.

Summary of Request: Washington State University Long Beach Research Unit is applying for two EUPs for the use of Imidacloprid to investigate the efficacy and nontarget effects of the pesticide against burrowing shrimp in oyster and manila clam beds in Willapa Bay and Grays harbor, Washington state. For 86414EUPR, the total quantity of product (Nuprid 2F, EPA Reg. No. 228484, containing 21.4% liquid imidacloprid) to be used is up to 80 pounds of active ingredient on up to 100 acres. For 86414EUPe, the total quantity of product (Mallet 0.5G, EPA Reg. No. 228501, containing 0.5% granular imidacloprid) to be used is up to 300 pounds of active ingredient on up to 30 acres.

A copy of the applications and any information submitted is available for public review in the docket established for these EUP applications as described under ADDRESSES.

Following the review of the applications and any comments and data received in response to this solicitation, EPA will decide whether to issue or deny the EUP requests, and if issued, the conditions under which it is to be conducted. Any issuance of EUPs will be announced in the Federal Register.

## List of Subjects

Environmental protection, Experimental use permits.

Dated: November 12, 2009.

Lois Rossi,

Director, Registration Division, Office of Pesticide Programs. [FR Doc. E928152 Filed 11/23/09; 8:45 am]  
BILLING CODE 656050S

## FOR FURTHER INFORMATION CONTACT

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Federal Register: November 24, 2009 (Volume 74, Number 225)

FR Doc E9-28152

DOCID: fr24no09-48

**ENVIRONMENTAL PROTECTION AGENCY**

**Environmental Protection Agency**

EPA ID: [EPA-HQ-OPP-2009-0660; FRL-8797-5]

**NOTICE: NOTICES**

**DOCID: fr24no09-48**

**DOCUMENT ACTION: Notice.**

**SUBJECT CATEGORY:**

Pesticide Experimental Use Permits; Receipt of Applications; Comment Requests

**DATES: Comments must be received on or before December 24, 2009.**

**DOCUMENT SUMMARY:**

This notice announces EPA's receipt of applications 86414-EUP- E and 86414EUPR from Washington State University Long Beach Research Unit requesting experimental use permits (EUPs) for the pesticide Imidacloprid. The Agency has determined that the permits may be of regional and national significance. Therefore, in accordance with 40 CFR 172.11 (a), the Agency is soliciting comments on these applications.

**SUMMARY:**

Pesticide Experimental Use Permits; Receipt of Applications; Comment Requests

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**SUPPLEMENTAL INFORMATION**

**I. General Information**

**A. Does this Action Apply to Me?**

This action is directed to the public in general. This action may, however, be of interest to those persons who are or may be required to conduct testing of chemical substances under the Federal Food, Drug, and Cosmetic Act (FFDCA) or the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Since other entities may also be interested, the Agency has not attempted to describe all the specific entities that may be affected by this action. If you have any questions regarding the applicability of this action to a particular entity, consult the person listed under FOR FURTHER INFORMATION CONTACT.

**B. What Should I Consider as I Prepare My Comments for EPA?**

1. Submitting CBI. Do not submit this information to EPA through regulations.gov or email. Clearly mark the part or all of the information that you claim to be CBI. For CBI information in a disk or CDROM that you mail to EPA, mark the outside of the disk or CDROM as CBI and then identify electronically within the disk or CDROM the specific information that is claimed as CBI. In addition to one complete version of the comment that includes information claimed as CBI, a copy of the comment that does not contain the information claimed as CBI must be submitted for inclusion in the public docket. Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR part 2.

2. Tips for preparing your comments. When submitting comments, remember to:

i. Identify the document by docket ID number and other identifying information (subject heading, Federal Register date

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460  
OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

PC Code: 129099  
DP Barcode: D368313 and D368315  
Date: November 20, 2009

**MEMORANDUM**

**SUBJECT:** Experimental Use Permit for Imidacloprid Products NUPRID 2F and MALLET 0.5G for Control of Burrowing Shrimp on Oyster Beds in Washington State.

**FROM:** N.E. Federoff, Wildlife Biologist  
Ron Parker, Senior Environmental Engineer  
Environmental Risk Branch V  
Environmental Fate and Effects Division (7507P)

*[Signature]*  
*Ronald D. Parker Nov 25, 2009*

**THROUGH:** Mah Shamim, Branch Chief  
Environmental Risk Branch V  
Environmental Fate and Effects Division (7507P)

*M. Shamim* 12/01/09

**TO:** Joanne Edwards, Risk Manager Reviewer  
John Herbert, Risk Manager 07  
Registration Division (7505P)

EFED has conducted a review of the proposed new uses for Imidacloprid. The assessment and conclusions are as follows:

**Background**

Washington State University (WSU) is applying for an EUP (Experimental Use Permit) for NUPRID 2F and MALLET 0.5G to control borrowing shrimp on oyster beds. The EUP is to be used to investigate the efficacy and non-target effects of Imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor in the State of Washington. The new products are for aquatic treatment only. The proposed use period is May through October 2010. NUPRID 2F (21.4% ai) is to be applied to 80 acres. MALLET 0.5G (0.5% ai) is to be applied to 30 acres. The highest current application rate for crops is 0.5 lbs ai/A. Under the current EUP, 1-2 lbs ai/A is proposed to be used on some of the acreage to be treated. All aerial and ground based applications must be made to exposed beds at low tide. Subsurface injections from a floating platform must be made to beds under water. A 200 ft buffer zone must be maintained for aerial applications and a 50 ft buffer zone must be maintained for hand held applications. All EUP restrictions should be followed. All spray drift management precautions and restrictions should also be



followed.

### Conclusions

EFED's screening assessment suggests that exposure from this compound to an estuarine/marine system supports the intent of this EUP. Acute and chronic risk is demonstrated to estuarine/marine invertebrates (burrowing shrimp) in the sediment pore water (acute RQ = 5.20; chronic RQ = 19.50). This demonstrates efficacy of Imidacloprid to the borrowing shrimp. This assessment also demonstrates very low risk to the surrogate eastern oyster species (acute RQ < 0.0013). Risks within the Bay will likely be localized to the target area. Imidocloprid is shown to be less toxic to estuarine mollusks than it is to estuarine invertebrates by several orders of magnitude.

### Environmental Fate of Imidacloprid

A summary of key environmental fate parameters (as determined for aquatic exposure modeling) is provided in Table 1. The major routes of dissipation for imidacloprid appear to be photolysis and anaerobic aquatic metabolism. Imidacloprid appears to be stable to aerobic soil metabolism. The chemical is mobile and is a major concern for ground waters, where there have been detections. Its transformation product imidacloprid guanidine is of concern as well. Imidacloprid may readily runoff dissolved in water and reach adjacent bodies of water. Since the chemical appears to be persistent under aerobic soil metabolism, imidacloprid may be available for runoff for periods exceeding one season. Potentially important environmental degradates include:

- 1) imidacloprid guanidine, 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinimine {Alias NTN 38014, NTN 33823}
- 2) imidacloprid olefin, 1-[(6-chloro-3-pyridin1yl)methyl]-1,3-dihydro-2H-imidazol-2-imine
- 3) imidacloprid urea, 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinone.{ NTN 33519}.

It appears that photolysis plays an important role in the dissipation of imidacloprid, both in aqueous solution (half-life 0.2 days) and on soil (half-life 39 days). Another route of transformation that appears to be important for imidacloprid is anaerobic aquatic metabolism (half-life 27 days), with the formation of imidacloprid guanidine (66% at 249 days; 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidinimine {Alias NTN 38014, NTN 33823}), a compound that appeared to be very persistent. Imidacloprid is very persistent under aerobic soil metabolism conditions (half-lives were 660, 188, 248 and 341 days in four soils).

Based on its  $K_{OC}$  values, imidacloprid would have medium mobility, with  $K_{OC}$ s ranging from 161 to 256 (based on nine soils, five domestic and four foreign). However, based on its  $K_{ads}$  values, it appears that imidacloprid is mobile and has the potential to leach to subsurfaces. The  $K_{ads}$  range is 0.96-4.76 for the same nine soils. On the other hand, imidacloprid guanidine appears to be less mobile than the parent imidacloprid ( $K_{OC}$  range 327-942;  $K_{ads}$  range 0.76-14.20).

Due to the very low octanol/water partition coefficient of imidacloprid, it is not expected to bioaccumulate in fish and the data requirement was waived.

Five terrestrial field dissipation studies confirm the findings in the laboratory, that under aerobic soil metabolism conditions, imidacloprid persists substantially. The half-lives were as follows: >365, >>365, 146, 107, and >120 days.

**Table 1.** Imidacloprid environmental fate parameters (as used for aquatic exposure modeling input).

Parameter	Input	Source
Solubility (ppm)	580	Product chemistry submissions
Molecular weight	255.66	<a href="http://chemfinder.cambridgesoft.com">http://chemfinder.cambridgesoft.com</a> and Product Chemistry submissions.
Vapor Pressure (mm Hg or torr), 20 C	1.5E-09	Product chemistry submissions; Miles Technical & Safety Information sheet, March, 1992.
Henry's Law Constant (atm m <sup>3</sup> mol <sup>-1</sup> )	4.0E-12	Registrant. Unable to locate original submission. SRC PhysProp Database lists as 1.65E-15 atm-m <sup>3</sup> /mole at 25 C as an estimated value apparently calculated from the vapor pressure and water solubility.
Hydrolysis t <sub>1/2</sub> @ pH 7 (days)	Stable	MRID 42055337
Aerobic soil t <sub>1/2</sub> (days)	520	MRIDs 452393-01, 02, 42073501; 90% upper bound confidence limit of mean
Aerobic aquatic t <sub>1/2</sub> (days)	1040	2x the aerobic soil input value, per EFED guidance document
Photolysis t <sub>1/2</sub> in water (days)	0.2 to 39	Input guidance & MRIDs 42256376; 42256377; with consideration of persistence in irradiated water in ecotoxicity studies.
Organic carbon partition coefficient - K <sub>oc</sub> (mL/g)	178	MRIDs 425208-01 and 420553-38
Partition coefficient - K <sub>d</sub> (mL/g)	2.4	Willapa Bay Study

### Exposure Assessment in Willapa Bay

OPP has evaluated exposure data from two studies of Imidacloprid use for control of burrowing shrimp in Willapa Bay. The first is by Felsot and Rupert (2002).

In this study, water and sediment were collected directly in the treated plots or at various distances along a westerly transect from the plots. To establish transects, the center of each plot was located and personnel walked to assigned distances in the direction of tidal flow by following the flow lines left in the sandy sediment at low tide. Water samples were collected as the tide was coming in, and sediment samples were collected during low tide after the sediment was exposed. For this study, imidacloprid dissipation was monitored as the tide was rising in Willapa Bay. Four weeks after application, additional water samples were collected directly above the treated plots.



Over 99% of applied material dissipated from small plots within 24 h, but residues near the analytical detection limit were found in sediments 28 days later. At a distance of 152 meters along a transect from the plot in the direction of tidal flow, imidacloprid residues in water peaked within 10 minutes after initiation of tidal flow. Within 30 minutes, Imidacloprid residues were not detected, nor were residues detected in the water any time over the next month after application.

Within 15 meters from the edge of treated plots, average imidacloprid residues peaked at 17.7 µg/L (0.017 mg/L) when the incoming water was 2 inches deep. At a distance of 152 meters from the treated plot, Imidacloprid was detected (average of 1.0 µg/L) about five minutes after tidal flow started but quickly dissipated to below detection levels as the tide continued to rise. Twenty-four hours later, Imidacloprid was not detected in water sampled directly over the plots, nor was any detected 28 days after application either above or outside of the plot (maximum distance monitored was 152 m).

Plots were treated for burrowing shrimp control and then residues were monitored in sediment for 28 days. Initial concentration after application was 0.461 mg/kg dry weight. The half-life was less than 1 day, and 28 days later residues were still detectable (0.005 mg/kg) in sediments over the treated area. Within one day, residues in treated plots dropped to 0.0164 mg/kg and were not detected after 28 days (limit of detection at 0.0025 mg/kg). Imidacloprid rapidly dissipates from water by aqueous photolysis (half-life of 0.2 days) but is stable to hydrolysis at pH 7.

Chronic dry weight sediment concentration values (21 and 60 averages) are calculated by averaging the daily measured values with the interpolated daily values between them (assuming the concentration is zero at day 60). Chronic pore water concentration values are calculated from the dry weight values based on an assumption of equal volumes of water and solids in the sediment (OPP Standard Pond) and a K<sub>d</sub> of 2.4 for Imidacloprid. See Table 2.

**Table 2.** Willapa Bay Sediment Concentrations

	Sediment Concentration (mg/kg (ppm) dry weight)	Calculated Concentration (Pore Water: mg/L (ppm))
Initial (Day 0)	0.461 (measured)	0.1921 (calculated)
(Day 1)	0.0164 (measured)	0.0068 (calculated)
(Day 14)	0.00267 (measured)	0.0011 (calculated)
(Day 28)	0.00472 (measured)	0.0020 (calculated)
(21-day Average)	0.0281 (calculated)	0.0117 (calculated)
(60-day Average)	0.0118 (calculated)	0.0049 (calculated)

The second study was conducted using small plot trials during 2006 – 2008 with Imidacloprid (Admire 1.6F, Bayer Corp.; Imida 2F, Etigra) and is the one submitted with this EUP request. Imidacloprid was applied aerially using helicopters to 7 commercial shellfish beds on July 2. Experimental beds were proposed by grower collaborators and selected based on degree of shrimp infestation, size, and proximity to untreated areas or beds treated with Sevin. A 20 acre bed located near the mouth of the North River (A90)

had been fallow for at 12 years, had a moderate to heavy shrimp infestation and was isolated from other shellfish beds, so provided a good site to study both efficacy and non-target impact to salmonids. A 10 acre bed near the mouth of the Cedar River (A40) was also used as a site to assess both non-target impact and efficacy. In this study, water was sampled for analysis of Imidacloprid concentration directly on the bed of three beds and in the adjacent channels of two beds. On-bed samples were taken by grab near the center of the bed, initially when depth of the in-coming tide reached six inches and on subsequent high tides at mid-depth of the water column. In-channel grab samples were taken at both maximum low and high tides at mid-depth of the water column.

Concentrations of Imidacloprid sampled over the beds dropped precipitously between 1 and 6 hours after treatment and were not detected afterward. At one hour concentrations immediately over the bed were 0.120 ppm, 0.040 ppm and 0.040 ppm at plots 90, 40 and 163 respectively.

Water concentrations in channels adjacent to two plots were recovered at 6 hours (0.000015 ppm), at 24 hours (0.00009 ppm), at 49 hours (0.00006 ppm) and at 74 hours (0.00003 ppm) after treatment (plot 90) and at 24 hours (0.0003 ppm), at 49 hours (0.00009 ppm) and at 74 hours (0.00006 ppm) after treatment (plot 40). (Method Reporting Limit = 0.00002 ppm). These timings were synchronized to the high tides.

Measured Imidacloprid concentrations in the water directly above the treated beds as the tidal flow begins are extremely variable, dissipate within 30 minutes to a few hours in both studies and are not useful for risk assessment. In the second study, however, there were detections in the channels adjacent to the beds for up to three days (73 hours). Average concentrations in these adjacent channels are presented in Table 3 below.

**Table 3.** Average Imidacloprid Water Column Concentrations in Channels Adjacent to Treated Beds (Plots)

Time After Application	Water Column Concentrations (ppm)
(Day 0: 6 hours)	0.000015 (Plot 90 Only)
(Day 1: 24 hours)	0.000195
(Day 2: 49 hours)	0.000075
(Day 3: 73 hours)	0.000045

### **Risks to Terrestrial organisms**

No risks to terrestrial organisms are expected because the proposed uses are all in aquatic areas. No exposure should occur under the subsurface application method. Aerial application is made to exposed beds at low tide. These areas will be submerged later in the day at high tide. Any effects, if they occur at all, will likely be very much localized due to the small acreages under the current EUP and that the area will be submerged soon after application.

Acute toxicity studies with honeybees show that imidacloprid is very highly toxic to non-target insects ( $LD_{50}$  = 0.0039 - 0.078  $\mu$ g/bee). This is a concern for pollinators because



imidacloprid is a systemic pesticide which has been shown to translocate into the nectar and pollen of crop plants grown from treated seed. Studies with ornamental plants have shown that imidacloprid may also translocate into plant parts when the chemical is applied to the soil around the base of the plants. In these studies with ornamentals, detectable residues were found in flowers and leaves as long as 540 days after application to the soil. However, under the current application, risks to bees should be low since it is an aquatic use and not near bee habitats.

### Risks to Aquatic organisms

EECs were developed from the study of imidacloprid use in Willapa bay. EFED's screening assessment suggests that exposure from this compound to an estuarine/marine system could result in acute and chronic risk to estuarine/marine invertebrates in the sediment pore water (acute RQ = 5.20; chronic RQ = 19.50). Imidocloprid is less toxic to estuarine mollusks than it is to estuarine invertebrates by several orders of magnitude. This assessment also demonstrates very low risk to the surrogate eastern oyster species (Acute RQ < 0.0013). Risks within the bay will likely be localized to the target area. Risks to freshwater fish or invertebrates were not assessed because the product will not be used in those areas.

Acute and chronic RQ's for evaluating toxic risk of imidacloprid exposure to estuarine/marine fish & invertebrates in pore water. RQ's are based on the sheepshead minnow (*Cyprinodon variegatus*)  $LC_{50}$  = 163 ppm, NOAEC = 2.3 ppm<sup>1</sup> and mysid shrimp (*Mysidopsis bahia*)  $EC_{50}$  = 0.037 ppm, NOAEC = 0.0006 ppm and Eastern Oyster ( $EC_{50}$  > 145 ppm).

Use	Endpoint	Surrogate	$EC_{50}$ (ppm)	NOAEC (ppm)	EEC Peak (ppm)	EEC 21 & 60-Day Ave. (ppm)	Acute RQ (EEC/ $LC_{50}$ )	Chronic RQ (EEC/ NOAEC)
Aquatic Pore Water	Estuarine/ Marine Fish	Sheepshead Minnow	163	2.3	0.1921	0.0049	0.0012	0.0021
Aquatic Pore Water	Estuarine/ Marine invertebrate	Mysid Shrimp	0.037	0.0006	0.1921	0.0117	5.20	19.50
Aquatic Pore Water	Estuarine/ Marine invertebrate	Eastern Oyster	>145	N/A	0.1921	N/A	< 0.0013	N/A

<sup>1</sup> Extrapolated value using an acute/chronic ratio from freshwater fish

Imidacloprid exposure is not expected to result in direct acute and chronic toxic effects to fish. Secondary adverse effects (fish life stage development) and adverse effects at the ecosystem level both to the organisms themselves as well as producing food chain and population disruptions are also unlikely due to the limited extent of the applications within the bays. Impacts of diminished invertebrate diversity on ecosystem integrity have not been explicitly evaluated but are also believed to be minimal. The rate of invertebrate recovery and/or the impact of decreased invertebrate diversity on higher trophic levels are an uncertainty but are likely to be minimal due to the small scope of the proposed use..

### Risks to Endangered Species

Endangered estuarine invertebrates living in the pore water may be adversely affected from exposure to imidacloprid under this EUP. However, there were no estuarine invertebrates listed for that area in the EFED database.

### ***Probit Slope Analysis***

The probit slope response relationship is evaluated to calculate the chance of an individual event corresponding to the listed species acute LOCs. If information is unavailable to estimate a slope for a particular study, a default slope assumption of 4.5 is used as per original Agency assumptions of typical slope cited in Urban and Cook (1986).

### **Aquatic Species**

Acute toxicity studies for imidacloprid did provide raw data and estimates of slopes for most fish and invertebrate species. A default slope of 4.5 was used for freshwater fish. Based on this slope, the corresponding estimate chance of individual mortality following exposure is 1 in  $4.17 \times 10^8$ . Analysis of raw data from the aquatic acute toxicity studies provided slopes of 1.69 for freshwater invertebrates, 4.21 for estuarine/marine invertebrates and 6.82 for estuarine/marine fish. Based on these slopes, the corresponding estimate chance of individual mortality following imidacloprid exposure is 1 in 71.7 for freshwater invertebrates, 1 in  $4.62 \times 10^7$  for estuarine/marine invertebrates and 1 in  $1 \times 10^{16}$  for estuarine/marine fish.

### **Incident Reports**

The Agency's Ecological Incident Information System (EIIS) does contain reports of damage or adverse effects to non-target organisms attributed to the use of imidacloprid. There are incidents involving imidacloprid that have been noted reflecting lawn use and effects to non-target organisms: 1) surfaced dead grubs appeared to have been eaten by birds, resulting in the death of several young and adult robins; 2) possible runoff event from a lawn resulted in the death of 3,000 crayfish in a near-by stream; 3) "mad bee" disease in France; 4 & 5) lawn grass chemically burned by the application of the compound; and 6 & 7) bee kills

#I007257-001 A private citizen of Myerstown, Pa. reported watering in pesticide (GrubEx ) and then found that grubs had surfaced a couple of days later. He was very concerned to see that the birds that fed on the grubs died.

#I007892-007 Turf application resulted in possible runoff into McKenna Creek (Columbus, Ohio) killing about 3,000 crawfish. Pesticide application was made on 7/22, slight rain event occurred on 7/22 (0.01 inches) and on 7/23 (0.09 inches). On July 23 dead crawfish were found. Water samples taken two days after the incident showed imidacloprid residues at 0.17, 0.11, and 1.3 ppb. In all likelihood the initial concentration was much higher. Water samples also detected metolachlor residues.

#I010775-001 Protest by the National Union of French Beekeepers have targeted GAUCHO, made by Bayer AC. This product along with REGENT TS (fipronil) was used to coat sunflower seeds for protection against insects. The French Farm Ministry suspended use of GAUCHO over the concerns about the aberrant disorientated behavior ("mad bee disease") of honey bees that had been associated with the sunflower crop that had originated from the coated seeds. Imidacloprid residues were found in the nectar.



#I009445-035 September 1999, complaint from resident in Assonet, MA. Home owner applied GrubEx Season-Long Grub Control to his lawn in June. He claims that 50% of the lawn burned.

#I009445-036 Resident in Brooklyn, NY applied GrubEx Season-Long Grub Control to his lawn and the entire lawn turned brown.

# I020700-001 Bayer reported bee kill.

#I021017 August 2009, bee kills reported after application to Linden trees in Pittsburgh PA. Bee deaths ceased when trees stopped blooming.

A lack of reported incidents does not necessarily mean that such incidents have not occurred. In addition, incident reports for non-target plants and animals typically provide information on mortality events only. Reports for other adverse effects, such as reduced growth or impaired reproduction, are rarely received.

## Toxicity

Measures of ecological effects and exposure for Imidacloprid.

<i>Assessment Endpoint</i>		<i>Surrogate Species and Measures of Ecological Effect<sup>1</sup></i>	<i>Measures of Exposure</i>
Birds <sup>2</sup>	Survival	House sparrow acute oral LD <sub>50</sub> = 41.0 mg/kg (2.5G) (MRID 420553-09) Quail acute oral LD <sub>50</sub> = 152.3 mg/kg (MRID 420553-08) Mallard duck acute oral LC <sub>50</sub> >4797 ppm (MRID 420553-11) Bobwhite acute dietary LC <sub>50</sub> = 1536 ppm (MRID 420553-10)	Maximum residues on food items
	Reproduction and growth	Bobwhite chronic reproduction NOAEC = 36 ppm (MRID 420553-12) Mallard chronic reproduction NOAEC = 47 ppm (MRID 434665-01)	Maximum residues on food items
	Survival	Laboratory rat acute oral LD <sub>50</sub> = 424 mg/kg (MRID 420553-31)	Maximum residues on food items
	Reproduction and growth	Laboratory rat oral reproduction chronic NOAEC = 250 ppm (MRID 422563-40)	Maximum residues on food items
Freshwater fish <sup>3</sup>	Survival	Bluegill sunfish acute LC <sub>50</sub> >105 ppm (MRID 420553-14) Rainbow trout acute LC <sub>50</sub> >83 ppm (MRID 420553-15)	Peak EEC <sup>4</sup>
	Reproduction and growth	Rainbow trout chronic (early life-stage) NOAEC=1.2 ppm and LOAEC=2.5 ppm (MRID 420553-20)	60-day average EEC <sup>4</sup>
Freshwater Invertebrates	Survival	Midge acute EC <sub>50</sub> = 0.069 ppm (MRID 422563-04)	Peak EEC <sup>4</sup>
	Reproduction and growth	Water flea chronic (life cycle) NOAEC = 1.3 ppm LOAEC = 3.6 ppm (MRID 420553-21)	21-day average EEC <sup>4</sup>

<i>Assessment Endpoint</i>		<i>Surrogate Species and Measures of Ecological Effect<sup>1</sup></i>	<i>Measures of Exposure</i>
Estuarine/ Marine fish	Survival	Sheepshead minnow acute LC <sub>50</sub> = 163 ppm (MRID 420553-18)	Peak EEC <sup>4</sup>
	Reproduction and growth	(no data)	60-day average EEC <sup>4</sup>
Estuarine/ Marine Invertebrates	Survival	Eastern oyster acute EC <sub>50</sub> >145 ppm (MRID 422563-05) Mysid shrimp acute LC <sub>50</sub> = 0.037 ppm (MRID 420553-19)	Peak EEC <sup>4</sup>
	Reproduction and growth	Mysid chronic NOAEL > 0.0006 ppm and LOAEC = 0.0013 (MRID 420553-22)	21-day average EEC <sup>4</sup>
Terrestrial Plants <sup>5</sup>	Survival and growth	(no data)	Estimates of runoff and spray drift to non-target areas
Insects	Survival	Honeybee acute contact LD <sub>50</sub> = 0.0039 ug/bee (MRID 422730-03)	Maximum application rate
Aquatic Plants and Algae	Survival	Green algae EC <sub>50</sub> > 10 ppm (MRID 422563-74)	Peak EEC

<sup>1</sup> If species listed in this table represent most commonly encountered species from registrant-submitted studies, risk assessment guidance indicates most sensitive species tested within taxonomic group are to be used for baseline risk assessments.

<sup>2</sup> Birds represent surrogates for amphibians (terrestrial phase) and reptiles.

<sup>3</sup> Freshwater fish may be surrogates for amphibians (aquatic phase).

<sup>4</sup> One in 10-year return frequency.

<sup>5</sup> Four species of two families of monocots - one is corn, six species of at least four dicot families, of which one is soybeans. LD<sub>50</sub> = Lethal dose to 50% of the test population; NOAEC = No observed adverse effect concentration; LOAEC = Lowest observed adverse effect concentration; LC<sub>50</sub> = Lethal concentration to 50% of the test population; EC<sub>50</sub>/EC<sub>25</sub> = Effect concentration to 50%/25% of the test population.



## **Appendix A. Bibliography**

Felsot A. S. and Rupert, J.R., 2002. Imidacloprid residues in Willapa Bay (Washington State) water and sediment following application for control of burrowing shrimp. J. Agric. Food Chem. 50: 4417-4423.

**Appendix B. Environmental Fate and Transport Studies and Toxicity Studies for Imidacloprid**

**161-1 Hydrolysis**

<b>MRID</b>	<b>Citation Reference</b>
42055337	Yoshida, H. (1989) Hydrolysis of NTN 33893: Lab Project No: 88011/ ESR: 99708. Unpublished study prepared by Nihon Tokushu Noyaku Seizo K.K. 34 p.

**161-2 Photodegradation-water**

<b>MRID</b>	<b>Citation Reference</b>
42256376	Anderson, C. (1991) Photodegradation of NTN 33893 in Water: Lab Project Number: 88010: 101956. Unpublished study prepared by Nitokuno, ESR, Yuki Institute. 128 p.

**161-3 Photodegradation-soil**

<b>MRID</b>	<b>Citation Reference</b>
42256377	Yoshida, H. (1990) Photodegradation of NTN 33893 on Soil: Lab Project Number: 88012/ESR: 100249. Unpublished study prepared by Nihon Tokushu Noyaku Seizo K. K. 42 p.

**162-1 Aerobic soil metabolism**

<b>MRID</b>	<b>Citation Reference</b>
42073501	Anderson, C.; Fritz, R.; Brauner, A. (1991) Metabolism of ?Pyridinyl-C 14-Methylene  NTN 33893 in Sandy Loam under Anaerobic Conditions: Lab Project Number: 101241; M1250187-4. Unpublished study prepared by Bayer Ag--Leverkusen. 82 p.
45239301	Anderson, C.; Fritz, R.; Brauner, A. (1992) Metabolism of (Pyridinyl-(carbon 14)-Methylene) NTN 33893 in Loamy Sand Soil BBA 2.2 under Aerobic Conditions: Lab Project Number: M 1250187-4. Unpublished study prepared by Miles Incorporated. 83 p.
45239302	Fritz, C. (1992) Degradation of (Pyridinyl-(carbon 14)-Methylene) NTN 33893 in Silt Soil HOEFCHEN under Aerobic Conditions: Lab Project Number: M 1250187-4. Unpublished study prepared by Bayer AG. 54 p.

**162-3 Anaerobic aquatic metabolism**

<b>MRID</b>	<b>Citation Reference</b>
42256378	Fritz, R.; Hellpointner, E. (1991) Degradation of Pesticides Under Anaerobic Conditions in the System Water/Sediment: Imidacloprid, NTN 33893: Lab Project Number: 1520205-5: 101346. Unpublished study prepared by Bayer AG, Leverkusen-Bayerwerk. 69 p.

**163-1 Leaching /adsorption /desorption**

<b>MRID</b>	<b>Citation Reference</b>
42055338	Fritz, R. (1988) Adsorption/Desorption of NTN 33893 on Soils: Lab Project



- 42055339 Number: M 1310231/1: 99199. Unpublished study prepared by Bayer Ag. 50 p.  
Fritz, R.; Brauner, ?. (1988) Leaching Behavior of NTN 33893 Aged in Soil:  
Lab Project Number: M 1210225/3: 99635. Unpublished study prepared by  
Bayer Ag. 45 p.
- 42520801 Williams, M.; Berghaus, L.; Dyer, D. (1992) Soil/Sediment Adsorption-  
desorption of (carbon 14)-Imidacloprid: Lab Project Number: N3182101.  
Unpublished study prepared by ABC Labs, Inc. 70 p.
- 42520802 Williams, M.; Berghaus, L.; Dyer, D. (1992) Soil/Sediment Adsorption-  
desorption of (carbon 14)-NTN-33823: Lab Project Number: N3182102.  
Unpublished study prepared by ABC Labs, Inc. 63 p.
- 43142501 Hellpointner, E. (1994) Degradation and Translocation of Imidacloprid (NTN  
33893) under Field Conditions on a Lysimeter: Lab Project Number: ME/6/95:  
M/1330351/6: 106426. Unpublished study prepared by Bayer AG, Institute for  
Metabolism Research. 74 p.
- 43315201 Hellpointner, E. (1994) Degradation and Translocation of Imidacloprid (NTN  
33893) under Field Conditions on a Lysimeter: Amendment to the Original  
Report: Project Nos. M 1330351-6; 106426-1. Unpublished study prepared by  
Bayer AG. 12 p.

#### 164-1 Terrestrial field dissipation

MRID	Citation Reference
42256379	Rice, F.; Judy, D.; Koch, D.; et al. (1991) Terrestrial Field Dissipation for NTN 33893 in Georgia Soil: Lab Project Number: N3022101: 101987. Unpublished study prepared by ABC Laboratories, Inc. 422 p.
42256380	Rice, F.; Judy, D.; Koch, D.; et al. (1991) Terrestrial Field Dissipation for NTN 33893 in Minnesota Soil: Lab Project Number: N3022103: 101988. Unpublished study prepared by ABC Laboratories, Inc. 510 p.
42256381	Rice, F.; Judy, D.; Koch, D.; et al. (1991) Terrestrial Field Dissipation for NTN 33893 in California Soil: Lab Project Number: N3022102: 101989. Unpublished study prepared by ABC Laboratories, Inc. 561 p.
42256382	Rice, F.; Schwab, D.; Noland, P.; et al. (1992) Terrestrial Field Dissipation in Turf for NTN 33893 in Georgia Soil: Lab Project Number: 393553: 102603. Unpublished study prepared by ABC Laboratories, Inc., and Miles Inc. 353 p.
42256383	Rice, F.; Judy, D.; Noland, P.; et al. (1992) Terrestrial Field Dissipation in Turf for NTN 33893 in Minnesota: Lab Project Number: 393543: 102604. Unpublished study prepared by ABC Laboratories, Inc., and Agri-Growth Research, Inc. 409 p.
42256384	Noland, P.; Koch, A. (1991) Analytical Method for the Determination of NTN 33893 in Soil Samples: Lab Project Number: 39272-2: 101984. Unpublished study prepared by ABC Laboratories, Inc. 82 p.
42256385	Noland, P.; Koch, A. (1991) Analytical Method for the Determination of NTN 33893 in Turf Samples: Lab Project Number: 39354-2: 101981. Unpublished study prepared by ABC Laboratories, Inc. 64 p.
42734101	Bachlechner, G. (1992) Dissipation of Imidacloprid in Soil Under Field Conditions: Lab Project Number: RA-2082/91: 103948. Unpublished study prepared by Miles Inc. 89 p.
44631501	Noland, P. (1996) NTN 33893 Freezer Storage Stability Study in Soil and Turf: Lab Project Number: 107369: N3022301: N3022303. Unpublished study prepared by Bayer Corporation: ABC Laboratories, Inc. 86 p.





**166-1 Ground water-small prospective**

<b>MRID</b>	<b>Citation Reference</b>
44790102	Dyer, D. (1999) Progress Report #5 and Study Termination Request: Imidacloprid (ADMIRE)--Small-Scale Prospective Ground-Water Monitoring Study, Montcalm County, Michigan, 1996: Lab Project Number: 5635.00: N3212401: N3212401-PR5. Unpublished study prepared by Bayer Corporation and Levine. Fricke.Recon, Inc. 92 p.
44790103	Dyer, D. (1999) Progress Report #4 and Study Termination Request: Imidacloprid (ADMIRE)--Small-Scale Prospective Ground-Water Monitoring Study, Montcalm County, Michigan, 1996: Lab Project Number: N3212401: N3212401-PR4: 5635.00. Unpublished study prepared by Bayer Corporation and Levine.Fricke.Recon, Inc. 307 p.
45094701	Dyer, D.; Helfrich, K. (1999) Progress Report #6: Imidacloprid (Admire)--Small-Scale Prospective Ground-Water Monitoring Study Montclm County, Michigan, 1996: Lab Project Number: N3212401: 5635.00: 109383. Unpublished study prepared by Bayer Corp. and LFR Levine. Fricke, Inc. 87 p.
45094702	Dyer, D.; Helfrich, K. (2000) Progress Report #7: Imidacloprid (Admire)--Small-Scale Prospective Ground-Water Monitoring Study Montclm County, Michigan, 1996: Lab Project Number: N3212401: 5635.00: 109596. Unpublished study prepared by Bayer Corp. and LFR Levine. Fricke, Inc. 80 p.
45094703	Lenz, M.; Helfrich, K. (2000) Imidacloprid (Admire)--Prospective Ground-Water Monitoring Study, California, Broccoli--Progress Report #12: Lab Project Number: 108939: H5034: N3212402. Unpublished study prepared by Bayer Corp. and LFR Levine. Fricke, Inc. 55 p.
45858201	Dyer, D.; Helfrich, K.; Billesbach, K. (2002) Imidacloprid--Small-Scale Prospective Ground-Water Monitoring Study, Montcalm County, Michigan, 1996: Lab Project Number: N3212401: 5635.00: CMXX-95-0229. Unpublished study prepared by Bayer Corporation, LFR Levine-Fricke, and Braun Intertec Corporation. 504 p.
45878701	Lenz, M.; Jackson, S.; Billesbach, K. (2002) Imidacloprid Prospective Groundwater Monitoring Study: Monterey County, California: Lab Project Number: N3212402: H5034: 110889. Unpublished study prepared by Bayer Corporation and Weber, Hayes & Associates. 813 p.

**Ecological Studies for Imidacloprid:****71-1 Avian Single Dose Oral Toxicity**

<b>MRID</b>	<b>Citation Reference</b>
42055308	Toll, P. (1990) Technical NTN 33893: An Acute Oral LD50 with Bob- white Quail: Lab Project Number: N3711702: 100059. Unpublished study prepared by Mobay Corp. 25 p.
42055309	Stafford, T. (1991) NTN 33893 2. 5G: An Acute Oral LD50 with House Sparrows (Passer domesticus): Lab Project No: N3711402: 101324. Unpublished study prepared by Mobay Corp. 23 p.
44059401	Hancock, G. (1996) NTN 33893 Technical: An Acute Oral LD50 with Mallards: (Final Report): Lab Project Number: 107354: N3710802. Unpublished study prepared by Bayer Corp. 32 p.

44457401 Schmuck, R. (1997) Acute Oral LD50 of Confidor WG 70 to Japanese Quail: (Final Report): Lab Project Number: 107904: E 293 1017-3: SXR/VW 178. Unpublished study prepared by Bayer AG Crop Protection. 35 p.

#### 71-2 Avian Dietary Toxicity

MRID	Citation Reference
42055310	Toll, P. (1990) Technical NTN 33893: Subacute Dietary LC50 with Bobwhite Quail: Lab Project Number: N3721702: 100241. Unpublished study prepared by Mobay Corp. 39 p.
42055311	Toll, P. (1991) Technical NTN 33893: A Subacute Dietary LC50 with Mallard Ducks: Lab Project Number: N3720801: 100238. Unpublished study prepared by Mobay Corp. 36 p.

#### 71-4 Avian Reproduction

MRID	Citation Reference
42055312	Toll, P. (1991) Technical NTN 33893: A One Generation Reproduction Study with Bobwhite Quail: Lab Project Number: N3741701: 1011203 . Unpublished study prepared by Mobay Corp. 114 p.
42055313	Toll, P. (1991) Technical NTN 33893: A One Generation Reproduction Study with Mallard Ducks: Lab Project Number: N3740801: 101205. Unpublished study prepared by Mobay Corp. 105 p.
42480502	Stafford, T. (1992) Technical NTN 33893: A One Generation Reproduction Study with Mallard Ducks: Lab Project Number: N3740802: 103813. Unpublished study prepared by Miles, Inc. 99 p.
43466501	Hancock, G. (1994) Effect of Technical NTN 33893 on Eggshell Quality in Mallards: Lab Project Number: N3740804: 106623. Unpublished study prepared by Miles Inc. 84 p.

#### 71-5 Simulated or Actual Field Testing

MRID	Citation Reference
42737101	Toll, P.; Fischer, D. (1993) Merit 0.62% Granular Insecticide: An Evaluation of Its Effects Upon Birds at Golf Courses in the Columbus, Ohio Vicinity: Lab Project Number: N3752302: 105002. Unpublished study prepared by Miles, Inc. 824 p.

#### 72-1 Acute Toxicity to Freshwater Fish

MRID	Citation Reference
42055314	Bowman, J.; Bucksath, J. (1990) Acute Toxicity of NTN 33893 To Blue gill ( <i>Lepomis macrochirus</i> ): Lab Project Number: 37860: 100348. Unpublished study prepared by Analytical Bio-chemistry Labs., Inc. 29 p.
42055315	Bowman, J.; Bucksath, J. (1990) Acute Toxicity of NTN 33893 to Rain bow Trout ( <i>Oncorhynchus mykiss</i> ): Lab Project Number: 37861: 100349. Unpublished study prepared by Analytical Bio-Chemistry Labs., Inc. 31 p.
42055316	Grau, R. (1988) The Acute Toxicity of NTN 33893 Technical to Rain- bow Trout ( <i>Salmo gairdneri</i> ) in a Static Test: Lab Project No: E 2800098-7: 101303. Unpublished study prepared by Bayer Ag. 18 p.



## 72-2 Acute Toxicity to Freshwater Invertebrates

MRID	Citation Reference
42055317	Young, B.; Hicks, S. (1990) Acute Toxicity of NTN 33893 To <i>Daphnia magna</i> : Lab Project Number: 37862: 10245. Unpublished study prepared by Analytical Bio-Chemistry Labs., Inc. 30 p.
42256303	England, D.; Bucksath, J. (1991) Acute Toxicity of NTN 33893 to <i>Hyalella azteca</i> : Lab Project Number: 39442: 101960. Unpublished study prepared by ABC Labs., Inc. 29 p.
43946601	Roney, D.; Bowers, L. (1996) Acute Toxicity of (carbon 14)-NTN 33823 to <i>Hyalella azteca</i> Under Static Conditions: Lab Project Number: 107315: N3823202. Unpublished study prepared by Bayer Corp. 34 p.
43946602	Bowers, L. (1996) Acute Toxicity of (carbon 14)-NTN 33823 to <i>Chironomus tentans</i> Under Static Conditions: Lab Project Number: 107316: N3823302. Unpublished study prepared by Bayer Corp. 30 p.
43946603	Dobbs, M.; Frank, J. (1996) Acute Toxicity of (carbon 14)-NTN 33519 to <i>Hyalella azteca</i> Under Static Conditions: Lab Project Number: 107148: N3823201. Unpublished study prepared by Bayer Corp. 31 p.
43946604	Dobbs, M.; Frank, J. (1996) Acute Toxicity of (carbon 14)-NTN 33519 to <i>Chironomus tentans</i> Under Static Conditions: Lab Project Number: 107311: N3823301. Unpublished study prepared by Bayer Corp. 35 p.
44558901	Bowers, L.; Lam, C. (1998) Acute Toxicity of 6-chloronicotinic acid (a metabolite of Imidacloprid) to <i>Chironomus tentans</i> Under Static Renewal Conditions: Lab Project Number: 96-B-123: 108127. Unpublished study prepared by Bayer Corporation. 24 p.

## 72-3 Acute Toxicity to Estuarine/Marine Organisms

MRID	Citation Reference
42055318	Ward, G. (1990) NTN-33893 Technical: Acute Toxicity to Sheepshead Minnow, <i>Cyprinodon variegatus</i> , Under Static Test Conditions: Lab Project Number: J9008023E: 100354. Unpublished study prepared by Toxikon Environmental Sciences. 36 p.
42055319	Ward, S. (1990) NTN-33893 Technical: Acute Toxicity to the Mysid, <i>Mysidopsis bahia</i> , Under Flow-Through Test Conditions: Lab Project Number: J9008023B/F: 100355. Unpublished study prepared by Toxikon Environmental Sciences. 46 p.
42256305	Wheat, J.; Ward, S. (1991) NTN 33893 Technical: Acute Effect on New Shell Growth of the Eastern Oyster, <i>Crassostrea virginica</i> : Lab Project Number: J9008023D: J9107005. Unpublished study prepared by Toxikon Environmental Sciences. 54 p.
42528301	Lintott, D. (1992) NTN 33893 (240 FS Formulation): Acute Toxicity to the Mysid, <i>Mysidopsis bahia</i> under Flow-through Conditions: Lab Project Number: J9202001: 103845. Unpublished study prepared by Toxikon Environmental Sciences. 43 p.

## 72-4 Fish Early Life Stage/Aquatic Invertebrate Life Cycle Study

MRID	Citation Reference
42055320	Cohle, P.; Bucksath, J. (1991) Early Life Stage Toxicity of NTN 33893

- Technical to Rainbow Trout (*Oncorhynchus mykiss*) in a Flow-through System: Lab Project Number: 38347: 101214. Unpublished study prepared by Analytical Bio-Chemistry Labs., Inc. 8 p.
- 42055321 Young, B.; Blake, G. (1990) 21-Day Chronic Static Renewal Toxicity of NTN 33893 To *Daphnia magna*: Lab Project No: 38346: 100247. Unpublished study prepared by Analytical Bio-Chemistry Labs., Inc. 84 p.
- 42055322 Ward, G. (1991) NTN 33893 Technical: Chronic Toxicity to the Mysid, *Mysidopsis bahia*, Under Flow-Through Test Conditions: Lab Project Number: J9008023G/H: 101347. Unpublished study prepared by Toxikon Environmental Sciences. 87 p.
- 42256304 Gagliano, G. (1991) Growth and Survival of the Midge (*Chironomus tentans*) Exposed to NTN 33893 Technical Under Static Renewal Conditions: Lab Project Number: N3881401: 101985. Unpublished study prepared by Mobay Corp. 43 p.
- 42480501 Gagliano, G. (1992) Raw Data and Statistical Analysis Supplement for Early Life Stage Toxicity of NTN 33893 to Rainbow Trout (*Oncorhynchus mykiss*): Lab Project Number: 38347. Unpublished study prepared by ABC Labs, Inc. 292 p.

#### 141-1 Honey bee acute contact

MRID	Citation Reference
42273003	Cole, J. (1990) The Acute Oral and Contact Toxicity to Honey Bees of Compound NTN 33893 Technical: Lab Project Number: 101321. Unpublished study prepared by RCC, Research and Consulting Company AG. 13 p.
42480503	Mayer, D.; Lunden, J.; Husfloen, M. (1991) Integrated Pest and Pollinator Investigations 1991 (Including Honey Bee Toxicity of NTN 33893): Lab Project Number: 103815. Unpublished study prepared by Miles, Inc. 13 p.

#### 141-2 Honey bee residue on foliage

MRID	Citation Reference
42480503	Mayer, D.; Lunden, J.; Husfloen, M. (1991) Integrated Pest and Pollinator Investigations 1991 (Including Honey Bee Toxicity of NTN 33893): Lab Project Number: 103815. Unpublished study prepared by Miles, Inc. 13 p.
42632901	Hancock, G.; Fischer, D.; Mayer, D.; et al. (1992) NTN 33893: Toxicity to Honey Bees on Alfalfa Treated Foliage: Lab Project Number: N3772902: 103938. Unpublished study prepared by Washington State University and Miles Residue Analysis Lab. 62 p.

#### 122-2 Aquatic plant growth

MRID	Citation Reference
42256374	Heimbach, F. (1989) Growth Inhibition of Green Algae ( <i>Scenedesmus suspicatus</i> ) Caused by NTN 33893 (Technical): Lab Project Number: 100098. Unpublished study prepared by Bayer Ag. 17 p.

#### 123-2 Aquatic plant growth

MRID	Citation Reference
42256375	Gagliano, G.; Bowers, L. (1991) Acute Toxicity of NTN 33893 Technical to the Green Algae ( <i>Selenastrum capricornutum</i> ): Lab Project Number: N3881601:



101986. Unpublished study prepared by Mobay Corp. 30 p.
- 44187101 Bowers, L. (1996) Toxicity of NTN 33893 2F to the Blue-Green Alga *Anabaena flos-aquae*: (Final Report): Lab Project Number: 107549: N3831401. Unpublished study prepared by Bayer Corp. 31 p.
- 44187102 Hall, A. (1996) Toxicity of NTN 33893 2F to the Freshwater Diatom *Navicula pelliculosa*: (Final Report): Lab Project Number: 107658: N3883401. Unpublished study prepared by Bayer Corp. 31 p.

#### Non-Guideline Studies

- 47303401 Doering, J.; Maus, C.; Anderson, C. (2004) Residues of Imidacloprid WG 5 in Blossom Samples of *Rhododendron* sp. (Variety Nova Zembla) after Soil Treatment in the Field - 2003. Project Number: G201796. Unpublished study prepared by Bayer CropScience Ag. 15 p.
- 47303402 Doering, J.; Maus, C.; Schoening, R. (2005) Residues of Imidacloprid WG 5 in Blossom and Leaf Samples of *Amelanchier* sp. after Soil Treatment in the Field - Application: 2003, Sampling: 2004 and 2005. Project Number: G201799, P672034512, AMELANCHIER/NTN33893WG5/DRENCH/NON/GLP. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 17 p.
- 47303403 Doering, J.; Maus, C.; Schoening, R. (2005) Residues of Imidacloprid WG 5 in Blossom Samples of *Cornus mas* after Soil Treatment in the Field - Application: 2003, Sampling: 2005. Project Number: G201801, P672034512, CORNUS/NTN33893WG5/DRENCH/NON/GLP. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 13 p.
- 47303404 Doering, J.; Maus, C.; Schoening, R. (2004) Residues of Imidacloprid WG 5 in Blossom Samples of *Rhododendron* sp. (Variety Nova Zembla) after Soil Treatment in the Field - Application: Spring 2003, Sampling 2003 and 2004. Project Number: G201806. Unpublished study prepared by Bayer CropScience and Bayer Ag, Institute of Product Info. & Residue Anal. 20 p.
- 47303405 Maus, C.; Schoening, R.; Doering, J. (2006) Assessment of Effects of Imidacloprid WG 70 on Foraging Activity and Mortality of Honey Bees and Bumblebees after Drenching Application under Field Conditions on Shrubs of the Species *Rhododendron catabiense grandiflorum* Surrounded by other. Project Number: G201808, P672054701, RHODO/MONITORING/FIELD/2005. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 25 p.
- 47303406 Maus, C.; Schoening, R.; Doering, J. (2007) Assessment of Effects of a Drench Application of Imidacloprid WG 70 to Shrubs of *Rhododendron* sp. and to *Hibiscus syriacus* on Foraging Activity and Mortality of Honeybees and Bumblebees Under Field Conditions. Project Number: FEILD/MONITORING/2006/RHODO/HIBI, P672064704, G201809. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 45 p.
- 47303407 Maus, C.; Schoening, R.; Doering, J. (2005) Assessment of Imidacloprid WG 5 in Blossom Samples of Shrubs of Different Sizes of the Species *Rhododendron* sp. after Drenching Application in the Field - Application 2004, Sampling 2005. Project Number: P672044712, G201813, RHODO05/NTN33893WG5/DRENCH/NON/GLP. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 18 p.
- 47303408 Doering, J.; Anderson, C.; Maus, C. (2004) Determination of the Residue Levels of Imidacloprid and Its Metabolites Hydroxy-Imidacloprid and Olefin-Imidacloprid in

- Leaves and Blossoms of Horse Chestnut Trees (*Aesculus hippocastanum*) After Soil Treatment - Application 2001 and Sampling 2002. Project Number: G201815. Unpublished study prepared by Bayer CropScience. 17 p.
- 47303409 Doering, J.; Anderson, C.; Maus, C. (2004) Determination of the Residue Levels of Imidacloprid and Its Metabolites Hydroxy-Imidacloprid and Olefin-Imidacloprid in Leaves and Blossoms of Horse Chestnut Trees (*Aesculus hippocastanum*) After Trunk Injection - Application 2001 and Sampling 2002. Project Number: G201817, P/672024504, MR/183/03. Unpublished study prepared by Bayer CropScience. 17 p.
- 47303410 Doering, J.; Maus, C.; Schoening, R. (2004) Residues of Imidacloprid WG 5 in Blossom Samples of Lime Trees (*Tilia europaea*) After Soil Treatment in the Field - Application: 2003, Sampling: 2004. Project Number: G201818, P672034513, TILIA/NTN33893WG5/DRENCH/NON/GLP. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 14 p.
- 47303411 Doering, J.; Maus, C.; Schoening, R. (2004) Residues of Imidacloprid WG 5 in Blossom and Leaf Samples of Apple Trees After Soil Treatment in the Field - Application: 2003, Sampling: 2004. Project Number: G201819, P672034511, MALUS/NTN33893WG5/DRENCH/NON/GLP. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 15 p.
- 47303412 Doering, J.; Maus, C.; Schoening, R. (2004) Residues of Imidacloprid WG 5 in Blossom Samples of Rhododendron sp. After Soil Treatment in the Field - Application: Autumn 2003, Sampling: 2004. Project Number: G201820, P672034514, RHODO/NTN33893WG5/DRENCH/NON/GLP. Unpublished study prepared by Bayer Ag, Institute of Product Info. & Residue Anal. and Bayer CropScience. 14 p.
- 47303413 Maus, C.; Anderson, C.; Doering, J. (2004) Determination of the Residue Levels of Imidacloprid and Its Relevant Metabolites in Nectar, Pollen and Other Plant Material of Horse Chestnut Trees (*Aesculus hippocastanum*) After Soil Treatment Application and Sampling 2001. Project Number: MAUS/AM021, E/370/2009/1. Unpublished study prepared by Bayer CropScience Ag. 23 p.
- 47303414 Maus, C.; Anderson, C.; Doering, J. (2004) Determination of the Residue Levels of Imidacloprid and Its Relevant Metabolites in Nectar, Pollen and Other Plant Material of Horse Chestnut Trees (*Aesculus hippocastanum*) After Trunk Injection Application and Sampling 2001. Project Number: MAUS/AM023, E/370/2057/4. Unpublished study prepared by Bayer CropScience. 27 p.

**The following studies are in review:**

- 47523401 Bonmatin, J.; Moineau, I.; Charvet, R.; et al. (2005) Behaviour of Imidacloprid in Fields. Toxicity for Honey Bees. P. 483-494 in Environmental Chemistry and Pollutants in Ecosystems by Lichtfouse, E., Schwartz-Bauer, J. and Robert, D. New York, NY: Springer
- 47523402 Suchail, S.; Guez, D.; Belzunces, L. (2001) Discrepancy Between Acute and Chronic Toxicity Induced by Imidacloprid and its Metabolites in *Apis mellifera*. Environmental Toxicology and Chemistry 20 (11) : 2482-2486.
- 47523403 Chauzat, M.; Faucon, J.; Martel, A.; et al. (2005) A Survey of Pesticide Residues in Pollen Loads Collected by Honey Bees In France. Entomological Society of America 99(2): 253-262.
- 47523404 Iwasa, T.; Motoyama, N.; Ambrose, J.; et al. (2003) Mechanism for the Differential Toxicity of Neonicotinoid Insecticides in the Honey Bee, *Apis mellifera*. Crop Protection 23(2004): 371-378.
- 47523405 Decourtye, A.; Armengaud, C.; Renou, M.; et al. (2003) Imidacloprid Impairs Memory and Brain Metabolism in the Honeybee (*Apis mellifera* L.). Pesticide Biochemistry and Physiology 78: 83-92.
- 47523406 Faucon, J.; Aurieres, C.; Drajnudel, P.; et al. (2005) Experimental Study on the Toxicity of Imidacloprid Given in Syrup to Honeybee (*Apis mellifera*) Colonies. Pest Management Science 61: 111-125.



- 47523407 Westwood, F.; Bean, K.; Dewar, A.; et al. (1998) Movement and Persistence of [Carbon 14] Imidacloprid in Sugar-Beet Plants Following Application to Pelleted Sugar-Beet Seed. *Pestic. Sci.* (52): 97-103.
- 47523408 Colin, M.; Bonmatin, J.; Moineau, I.; et al. (2004) A Method to Quantify and Analyze the Foraging Activity of Honey Bees: Relevance to the Sublethal Effects Induced by Systemic Insecticides. *Archives of Environmental Contamination and Toxicology* 47: 387-395.
- 47523409 Suchail, S.; Debrauwer, L.; Belzunces, L. (2003) Metabolism of Imidacloprid in *Apis mellifera*. *Pest Management Science* 60: 291-296.
- 47523410 Decourtyle, A.; Lacassie, E.; Phan-Delegue, M. (2003) Learning Performances on Honeybees (*Apis mellifera* L) are Differentially Affected by Imidacloprid According to the Season. *Pest Management Science* 59: 269-278.
- 47523411 Bonmatin, J.; Marchand, P.; Charvet, R.; et al. (2005) Quantification of Imidacloprid Uptake in Maize Crops. *Journal of Agricultural and Food Chemistry* 53: 5336-5341.



Re: EUP FR Notice  
Steven R. Booth to: Joanne Edwards

10/14/2009 12:16 PM

Joanne - I found the error.

Total for the Nuprid is 80 -- broken up into 30 ac @ 2 lb a.i./ac, 30 ac @ 1 lb, and 20 ac @ 0.5 lb

Also -- applicant should be Kim Patten only  
Not "Kim Patten, Ralph Cavalerri"  
I changed that for both Nuprid and Mallet forms.

Let me know if there is anything else.

Steve

----- Original Message -----

From: <Edwards.Joanne@epamail.epa.gov>  
To: "Steven R. Booth" <boothswa@comcast.net>  
Sent: Wednesday, October 14, 2009 8:42 AM  
Subject: EUP FR Notice


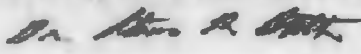
>  
> Steve- we have to publish Notice in FR on these oyster EUPS. You have  
> error on the 8570-17 form, shouldn't it be maximum 100 (60 ac@.....)  
>  
> I need you to confirm this, before I send for publishing, also you need  
> to resubmit 8570-17. with that correction (pdf ok)  
>  
> Joanne Edwards  
> EPA/OPPTS/OPP/RD/IRB  
> (703) 305-6736  
> edwards.joanne@epa.gov  
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WGHOGA 8570-17 Mallet October 2009.pdf WGHOHA 8570-17 Nuprid October 2009.pdf



Form Approved. OMB No. 2070-0040.

 <b>United States ENVIRONMENTAL PROTECTION AGENCY</b> Washington, DC 20460		OPP Identifier Number
Office of Pesticides Programs (7505C) <b>Application for Experimental Use Permit to Ship and Use a Pesticide for Experimental Purposes Only</b>		
<b>1. Type of Application</b> <input checked="" type="checkbox"/> <b>New</b> <input type="checkbox"/> <b>Amendment (See No. 2)</b> <input type="checkbox"/> <b>Extension (Give Permit Number below)</b>	<b>2. Briefly explain (attach a separate sheet if necessary)</b> This EUP is to be used to investigate the efficacy and nont-target effects of imidacloprid against burrowing shrimps in Willapa Bay and Grays Harbor, Washington.	
Permit Number		
<b>3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be issued (include Zip Code) (Type or Print)</b> Kim Patten, Extension Specialist, Professor Washington State University Long Beach Research and Unit 2907 Pioneer Road Long Beach WA 98631	<b>4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (include Zip Code) (Type or Print)</b> Nufarm Americas Inc. 150 Harvester Dr., Suite 200 Burr Ridge, IL 60527	
<b>EPA Company Number</b> 81959-22	<b>6. Is Product Registered with EPA?</b> <input type="checkbox"/> <b>No</b> <input checked="" type="checkbox"/> <b>Yes (Give Registration Number or File Symbol below)</b> Registration Number <u>EPA Reg. No. 228-484</u> File Symbol _____	
<b>5. Name of Product</b> Name of registered product: Nuprid 2F		
<b>7. Total Quantity of Product Proposed for Shipment/Use</b> Pounds of formulated product <u>415</u> Pounds of active ingredient <u>100</u>	<b>8. Acreage or Area to be Treated</b> maximum 80(30 ac@2 lb a.i./ac, 30 ac@1 lb a.i./ac, 20 ac@0.5 lb a.i./ac)	<b>9. Proposed Period of Shipment/Use</b> May 2010 – October 2010
<b>10. Places from which Shipped</b> Nufarm Inland Empire Dist 1211E St Helens ST STE B, Pasco, WA 99301	<b>11. Crop/Site to be Treated</b> Oysters and Manila Clams (Tapes philippinarum) Willapa Bay and Grays Harbor, Washington	
<b>12. Specify the name and number of the contact person most familiar with this application.</b> Kim Patten 360-642-2031 Steven R. Booth 360-867-4163	<b>13. Signature of Applicant or Authorized Firm Representative</b> 	
<b>14. Title</b> WBGHOGA IPM Coordinator		<b>15. Date Signed</b> 06/16/2009
<b>Certification</b>		
This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.		
I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.		
Below for EPA Use Only		
In any correspondence on this application, refer to this number		Received by: EPA-OPP Registration Division, Washington, DC 20460
Normal review time indicates that processing of this application should be completed by (date)		
Name of EPA Contact Person	Telephone Number	

## INSTRUCTIONS

Refer to 40 CFR 172 for regulations regarding experimental use permits. These regulations were published in the FEDERAL REGISTER on April 30, 1975 (40 FR 18780). Complete all (and only) numbered items on the application form. If an EPA Company Number (Item 2) has not previously been assigned, indicate "None," and a number will be assigned on your acknowledgment copy of the form. Third party applicants (those who will be testing another firm's registered product) need not complete Item 13. On the acknowledgment copy of this form, you will be assigned a File Number or Symbol for identification of this application. An expected completion date and the name of your EPA Contact will be entered. You may call your EPA Contact if you have not received your permit or a letter of explanation by the date indicated.

### Experimental Use Permit Data Submission

The following information must be submitted in triplicate and in detail (bound in removable sections A through G with margin tabs) for all new chemicals and many new products. For some new formulations, the information requested in Items C, D, E, and F may be included by reference to other formulations if adequate extrapolation may be made. Where the applicant requests permission to test a registered product, the information requested in Items B, E, F, and G below, along with the EPA Registration Number of the product, will usually suffice. Refer to 40 CFR 158.640 [53 FR 15993, May 4, 1988] for further information.

- A. A data sheet giving the chemical and physical properties of the chemical. A complete statement of the names and percentages by weight of each Active and Inert ingredient in the formulation to be shipped. This information will be handled as confidential material.
- B. One copy of the proposed label including directions for use necessary for evaluation of the product. Refer to 40 CFR 172.6 for minimum labeling requirements. In certain circumstances the experimental program or other supplemental labeling may be permissible in lieu of full labeling. In such cases, submit a full explanation as to how the labeling will be affixed to or accompany the container.
- C. Toxicity data or reference to available data on the toxicity of the pesticide including, where pertinent, data on the toxicity to fish and wildlife. Include a summary of this information. LD<sub>50</sub> values and results of eye irritation studies on the formulated product must be included.
- D. Residue data, where pertinent, on (a) food or feed commodities; (b) nonfood crops such as tobacco; and (c) foliage or other sites which may relate to worker hazard or adverse effects on the environment. Include a description of the analytical method(s) used and a summary of the data.
- E. Effectiveness data [required only if specified in Regulations 40 CFR 158.640, 53 FR 15993, May 4, 1988 and Registration Guidelines 40 CFR 158.202(i), 53 FR 15993, May 4, 1988].
- F. If the pesticide is to be tested in a manner involving food or feed, and an adequate tolerance is not established to cover the use, file a petition for a temporary tolerance with this Agency and forward three copies with this application. If appropriate tolerances are established already, cite applicable Regulation in Title 40 of the Code of Federal Regulations.
- G. Proposed Experimental Program:
  - (1) Give the qualifications and the names, addresses, and telephone numbers of the individuals (participants) who will supervise the experimental work.
  - (2) Name the States in which the pesticide will be used and the acreage to be treated in each State. Where "acreage" does not apply, give extent of testing per State in more appropriate terminology. Indicate separately any other State(s) to which the pesticide may be shipped for further distribution.
  - (3) Give the details of the proposed program including the types of target pests or organisms, the crops, animals, surfaces, materials, buildings, or sites of application to be treated and the major geographical areas where the material is to be used. For seasonal pests or crops, indicate the desired month for pesticide application to begin. Specify the use pattern, intended plot sizes, number of plots, number of replicates, dosage rates, methods of application, season of use (spring, summer, fall) and timing of application (preplant, postemergence, multiple (indicate pattern and number), etc.).
  - (4) List the objectives of the proposed program including, e.g., what type(s) of data will be collected during the testing period (performance, yield, phytotoxicity, environmental residue, etc.). Indicate your long-range testing plans, including how many years you expect to conduct experimental testing in support of registration of this use. This information will be helpful in evaluating the currently proposed program.
  - (5) Submit an explanation to justify the quantity of the material requested, including various parameters used to determine the quantity. Quantities authorized will be based on the program submitted and consideration of the types and amount of data required to support registration.
  - (6) Propose a suitable duration for the permit commensurate with the program. Any request for a period greater than 1 year must be adequately justified.
  - (7) State the method of disposition of any unused material left at the conclusion of the testing program.

### Paperwork Reduction Act Notice

The public reporting burden for this collection of information is estimated to average three quarters of an hour including time for reviewing instructions, gathering existing product sources and addresses, shippers to be used and addresses, and completing this instrument. Send comments regarding this estimate or any other aspect of this process, including suggestions for reducing the burden to: Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460; Office of Management and Budget, Paperwork Reduction Project (2070-0040), Washington, DC 20503.

NOTE: Applicant may retain last copy  
(04-14-93)





Re: WGHOGA FEUP application for imidacloprid use on Willapa Bay shellfish beds

Steven R. Booth to: Joanne Edwards  
Cc: "Tim Morris"

08/17/2009 09:06 PM

Hi Joanne,

Attached are slightly revised Attachments 1 & 2, the 850-17 forms for both the liquid and granular formulations, and Experimental Labels. The labels are revised to make Kim Patten, WSU, the permittee, rather than Ralph Calvalerri, also WSU, but really not very involved with this effort. The granular label is also revised regarding application directions and also I dropped out the reference to droplet size.

I am in the field tomorrow but can be reached via my cell phone: 360-952-5158. I doubt I will have time to check my email before 5 pm East Coast time, though.

Please let me know if there is anything else we need to do.

Thanks,

Steve Booth

----- Original Message -----

From: <Edwards.Joanne@epamail.epa.gov>

To: "Steven R. Booth" <boothswa@comcast.net>

Sent: Friday, August 14, 2009 9:09 AM

Subject: Re: WGHOGA FEUP application for imidacloprid use on Willapa Bay shellfish beds

> Great- next Tuesday is fine. when we put in FR, we must listed what  
> acreage etc, so make sure to resubmit the application forms.

>  
> Joanne Edwards  
> EPA/OPPTS/OPP/RD/IRB  
> (703) 305-6736  
> edwards.joanne@epa.gov

>  
>  
> From: "Steven R. Booth" <boothswa@comcast.net>

> To: Joanne Edwards/DC/USEPA/US@EPA

> Date: 08/14/2009 12:07 PM

> Subject: Re: WGHOGA FEUP application for imidacloprid use on Willapa  
> Bay shellfish beds

>  
>  
>  
>  
>  
>  
> Joanne,

> Six months is about what I figured. I will change the study plans to  
> make  
> them a bit more general -- I just cant list specific locations right  
> now,  
> but I can come up with a fairly narrow range of acreages -- and get that  
> to  
> you this afternoon or next week.  
>  
> Thanks for your heads up on this so they dont get stuck reviewing  
> something  
> that wont be.  
>  
> Steve  
>  
>  
> ----- Original Message -----  
> From: <Edwards.Joanne@epamail.epa.gov>  
> To: "Steven R. Booth" <boothswa@comcast.net>  
> Sent: Friday, August 14, 2009 8:25 AM  
> Subject: Re: WGHOGA FEUP application for imidacloprid use on Willapa Bay  
>  
> shellfish beds  
>  
>  
>> Steve- I just checked with John, the timeframe is six months, and  
>> that's how long it will take!  
>>  
>>  
>> Therefore, if you have any changes, then you need to get them to me  
> next  
>> week, since I need to get it into review. Also, John mentioned we  
>> should be doing notices of receipt in the Federal register for each of  
>> these EUPs.  
>>  
>> Joanne Edwards  
>> EPA/OPPTS/OPP/RD/IRB  
>> (703) 305-6736  
>> edwards.joanne@epa.gov  
>>  
>>  
>>  
>> From: "Steven R. Booth" <boothswa@comcast.net>  
>>  
>> To: Joanne Edwards/DC/USEPA/US@EPA  
>>  
>> Cc: John Hebert/DC/USEPA/US@EPA  
>>  
>> Date: 08/14/2009 10:57 AM  
>>  
>> Subject: Re: WGHOGA FEUP application for imidacloprid use on  
> Willapa  
>> Bay shellfish beds  
>>  
>>  
>>  
>>  
>>  
>> Thanks Joanne,  
>>  
>> Is there any chance whatsoever of getting this through in the next



>> month?  
>> If not, I should change the "Proposed Experimental Program" somewhat  
> to  
>> make  
>> it more general and applicable for next year. Specifically, I cannot  
>> say  
>> exactly where we would treat next year - but I could make a general  
>> study  
>> plan. I would also have to change the acreage amounts in the 850-17.  
>> That  
>> is, make them a range (e.g., 50 -- 80).  
>>  
>> If there is a good chance of thee application being approved in the  
> next  
>>  
>> month, then I would only have to change the plan slightly, as we  
> treated  
>> one  
>> of the proposed study sites under Meredith Laws 10 ac exemption.  
>>  
>> Seems to me we should shoot for next year??  
>>  
>> Steve  
>>  
>> ----- Original Message -----  
>> From: <Edwards.Joanne@epamail.epa.gov>  
>> To: "Steven R. Booth" <boothswa@comcast.net>  
>> Cc: <Hebert.John@epamail.epa.gov>  
>> Sent: Friday, August 14, 2009 7:36 AM  
>> Subject: Re: WGHOGA FEUP application for imidacloprid use on Willapa  
> Bay  
>>  
>> shellfish beds  
>>  
>>  
>>> Steve- I just received your applications for the two EUPS for  
>>> processing. I will put these into review (EFED) next week, but  
> before  
>>> doing so, I need to know if anything has changed, e.g.:  
>>>  
>>> the information on the forms 8570-17,  
>>>  
>>> the draft labels  
>>>  
>>> the justification in the June 30, 2009 letter  
>>>  
>>> the Attachments  
>>>  
>>> if so, I need you to let me know, and then email me the revised  
>>> documents.  
>>>  
>>>  
>>> Joanne Edwards  
>>> EPA/OPPTS/OPP/RD/IRB  
>>> (703) 305-6736  
>>> edwards.joanne@epa.gov  
>>>  
>>>  
>>>  
>>> From: "Steven R. Booth" <boothswa@comcast.net>  
>>>

>>> To: Joanne Edwards/DC/USEPA/US@EPA  
>>>  
>>> Date: 07/06/2009 04:43 PM  
>>>  
>>> Subject: Re: WGHOGA FEUP application for imidacloprid use on  
>> Willapa  
>>> Bay shellfish beds  
>>>  
>>>  
>>>  
>>>  
>>>  
>>> Thanks Joanne  
>>>  
>>> ----- Original Message -----  
>>> From: <Edwards.Joanne@epamail.epa.gov>  
>>> To: "Steven R. Booth" <boothswa@comcast.net>  
>>> Sent: Monday, July 06, 2009 1:29 PM  
>>> Subject: Re: WGHOGA FEUP application for imidacloprid use on Willapa  
>> Bay  
>>>  
>>> shellfish beds  
>>>  
>>>  
>>>> Steve- No, I haven't seen it. I am unfamiliar with the processing  
> of  
>>>> applications when they come in through the mailroom. I do know  
> they  
>>>> get sorted, and then undergo a screening process, before being  
>>> assigned  
>>>> to the PM team. John serves (or has served) on the screening  
>>> committee.  
>>>> He would have a better idea on the time it takes to get from  
> mailroom  
>>> to  
>>>> his next. He's the one that assigns the application to me! Sorry,  
> I  
>>>> can't be of much help, other than to tell you it will take some time  
>>> to  
>>>> get processed. We have to put it into review in EFED, and they have  
>> a  
>>>> certain period of time to complete a review. John also has that  
>>>> information on what time the Agency has to process an EUP  
>> application.  
>>>> Check with John.  
>>>>  
>>>> Joanne Edwards  
>>>> EPA/OPPTS/OPP/RD/IRB  
>>>> (703) 305-6736  
>>>> edwards.joanne@epa.gov  
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MALLET EXPERIMENTAL LABEL August.pdf WGHOGA FEUP Attachments 1 & 2 August 2009.pdf



WGHOGA 8570-17 Nuprid August 2009.pdf WGHOGA 8570-17 Mallet August 2009.pdf



NUPRID EXPERIMENTAL LABEL August 09.pdf





Form Approved. OMB No. 2070-0040.



United States  
**ENVIRONMENTAL PROTECTION AGENCY**  
 Washington, DC 20460

OPP Identifier Number

Office of Pesticides Programs (7505C)

**Application for Experimental Use Permit to Ship and  
 Use a Pesticide for Experimental Purposes Only**

**1. Type of Application**

New



Amendment (See No. 2)



Extension (Give Permit Number below)

Permit Number

**2. Briefly explain (attach a separate sheet if necessary)**

This EUP is to be used to investigate the efficacy and nontarget effects of imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor, Washington.

**3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be Issued (include Zip Code) (Type or Print)**

Kim Patten, Ralph Cavalieri, Extension Specialist, Professor  
 Washington State University Long Beach Research and Unit  
 2907 Pioneer Road  
 Long Beach WA 98631

**4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (include Zip Code) (Type or Print)**

Nufarm Americas Inc.  
 150 Harvester Dr., Suite 200  
 Burr Ridge, IL 60527

EPA Company Number 81959-22

**5. Name of Product**

Name of registered product: Nuprid 2F

**6. Is Product Registered with EPA?**

No



Yes (Give Registration Number or File Symbol below)

Registration Number EPA Reg. No. 228-484

File Symbol

**7. Total Quantity of Product Proposed for Shipment/Use**

Pounds of formulated product 415

Pounds of active ingredient 100

**8. Acreage or Area to be Treated**

maximum 80(60 ac@2 lb a.i./ac, 30  
 ac@1 lb a.i./ac, 10 ac@0.5 lb a.i./ac)

**9. Proposed Period of Shipment/Use**

May 2010 - October 2010

**10. Places from which Shipped**

Nufarm Inland Empire Dist  
 1211E St Helens ST STE B, Pasco, WA 99301

**11. Crop/Site to be Treated**

Oysters and Manila Clams (Tapes philippinarum)  
 Willapa Bay and Grays Harbor, Washington

**12. Specify the name and number of the contact person most familiar with this application.**

Kim Patten 360-642-2031  
 Steven R. Booth 360-867-4163

**13. Signature of Applicant or Authorized Firm Representative**
**14. Title**

WBGHOGA IPM Coordinator

**15. Date Signed**

06/16/2009

**Certification**

This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.

I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.

**Below for EPA Use Only**

In any correspondence on this application, refer to this number

Received by:  
 EPA OPP Registration Division,  
 Washington, DC 20460

Normal review time indicates that processing of this application should be completed by (date)

Name of EPA Contact Person

Telephone Number



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- A. A data sheet giving the chemical and physical properties of the chemical. A complete statement of the names and percentages by weight of each Active and Inert ingredient in the formulation to be shipped. This information will be handled as confidential material.
- B. One copy of the proposed label including directions for use necessary for evaluation of the product. Refer to 40 CFR 172.6 for minimum labeling requirements. In certain circumstances the experimental program or other supplemental labeling may be permissible in lieu of full labeling. In such cases, submit a full explanation as to how the labeling will be affixed to or accompany the container.
- C. Toxicity data or reference to available data on the toxicity of the pesticide including, where pertinent, data on the toxicity to fish and wildlife. Include a summary of this information. LD<sub>50</sub> values and results of eye irritation studies on the formulated product must be included.
- D. Residue data, where pertinent, on (a) food or feed commodities; (b) nonfood crops such as tobacco; and (c) foliage or other sites which may relate to worker hazard or adverse effects on the environment. Include a description of the analytical method(s) used and a summary of the data.
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- F. If the pesticide is to be tested in a manner involving food or feed, and an adequate tolerance is not established to cover the use, file a petition for a temporary tolerance with this Agency and forward three copies with this application. If appropriate tolerances are established already, cite applicable Regulation in Title 40 of the Code of Federal Regulations.
- G. Proposed Experimental Program:
  - (1) Give the qualifications and the names, addresses, and telephone numbers of the individuals (participants) who will supervise the experimental work.
  - (2) Name the States in which the pesticide will be used and the acreage to be treated in each State. Where "acreage" does not apply, give extent of testing per State in more appropriate terminology. Indicate separately any other State(s) to which the pesticide may be shipped for further distribution.
  - (3) Give the details of the proposed program including the types of target pests or organisms, the crops, animals, surfaces, materials, buildings, or sites of application to be treated and the major geographical areas where the material is to be used. For seasonal pests or crops, indicate the desired month for pesticide application to begin. Specify the use pattern, intended plot sizes, number of plots, number of replicates, dosage rates, methods of application, season of use (spring, summer, fall) and timing of application (preplant, postemergence, multiple (indicate pattern and number), etc.).
  - (4) List the objectives of the proposed program including, e.g., what type(s) of data will be collected during the testing period (performance, yield, phytotoxicity, environmental residue, etc.). Indicate your long-range testing plans, including how many years you expect to conduct experimental testing in support of registration of this use. This information will be helpful in evaluating the currently proposed program.
  - (5) Submit an explanation to justify the quantity of the material requested, including various parameters used to determine the quantity. Quantities authorized will be based on the program submitted and consideration of the types and amount of data required to support registration.
  - (6) Propose a suitable duration for the permit commensurate with the program. Any request for a period greater than 1 year must be adequately justified.
  - (7) State the method of disposition of any unused material left at the conclusion of the testing program.

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NOTE: Applicant may retain last copy  
(04-14-93)



**NUPRID 2F**

**FOR EXPERIMENTAL USE ONLY**

Experimental Use Permit Number:

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

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**Permittee:**

Kim Patten, Extension Specialist, Professor  
Washington State University Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach WA 98631

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**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine .....21.4%

OTHER INGREDIENTS: ..... 78.6%

TOTAL: ..... 100.0%

Contains 2 pounds of imidacloprid per gallon.

**KEEP OUT OF REACH OF CHILDREN**

**CAUTION – CAUCION**

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No.

FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>NOTE TO PHYSICIAN</b> No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS**  
**HAZARDS TO HUMANS AND DOMESTIC ANIMALS**  
**CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
  - Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
  - Shoes plus socks
  - Protective eyewear when working in a non-ventilated space
- Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Users must:**

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.  
**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 2.0 lb a.i./ac using the following properly calibrated application equipment:

- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~20 gpa.
- SpikeWheel™ spoke wheel subsurface injectors operated from a floating platform at ~20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500ft.
- For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.
- Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between June 1 and October 31.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within a one-and-a-half (1 1/2) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (1/4) of an inch high. Signs shall also state "Do Not Fish, Crab, or Clam". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

**SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.



### **Restrictions During Temperature Inversions**

Because the potential for spray drift is high during temperature inversions, do NOT make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing, which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

### **Importance of Droplet Size**

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

### **Mixing and Loading Requirements**

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

### **STORAGE AND DISPOSAL**

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Refer to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk through spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak incidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.





Form Approved. OMB No. 2070-0040.



United States  
**ENVIRONMENTAL PROTECTION AGENCY**  
 Washington, DC 20460

OPP Identifier Number

Office of Pesticides Programs (7505C)

**Application for Experimental Use Permit to Ship and  
 Use a Pesticide for Experimental Purposes Only**

**1. Type of Application**

New



Amendment (See No. 2)



Extension (Give Permit Number below)

Permit Number

**2. Briefly explain (attach a separate sheet if necessary)**

This EUP is to be used to investigate the efficacy and nontarget effects of imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor, Washington.

**3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be issued (include Zip Code) (Type or Print)**

Kim Patten, Ralph Cavalieri, Extension Specialist, Professor  
 Washington State University Long Beach Research and Unit  
 2907 Pioneer Road  
 Long Beach WA 98631

**4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (include Zip Code) (Type or Print)**

Nufarm Americas Inc.  
 150 Harvester Dr., Suite 200  
 Burr Ridge, IL 60527

EPA Company Number 81959-22

**5. Name of Product**

Name of registered product: Mallet 0.5G

**6. Is Product Registered with EPA?**

No



Yes (Give Registration Number or File Symbol below)

Registration Number EPA Reg. No. 228-501

File Symbol

**7. Total Quantity of Product Proposed for Shipment/Use**

Pounds of formulated product 5,000

Pounds of active ingredient 300

**8. Acreage or Area to be Treated**

maximum 30  
 (20 ac @ 1 lb a.i./ac + 10 ac @ 0.5 lb  
 a.i./ac)

**9. Proposed Period of Shipment/Use**

May 2010 - October 2010

**10. Places from which Shipped**

Nufarm Inland Empire Dist  
 1211E St Helens ST STE B, Pasco, WA 99301

**11. Crop/Site to be Treated**

Oysters and Manila Clams (Tapes philippinarum)  
 Willapa Bay and Grays Harbor, Washington

**12. Specify the name and number of the contact person most familiar with this application.**

Kim Patten 360-642-2031  
 Steven R. Booth 360-867-4163

**13. Signature of Applicant or Authorized Firm Representative**
**14. Title**

WBGHOGA IPM Coordinator

**15. Date Signed**

06/30/2009

**Certification**

This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.

I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.

**Below for EPA Use Only**

In any correspondence on this application, refer to this number

Received by:  
 EPA-OPP Registration Division,  
 Washington, DC 20460

Normal review time indicates that processing of this application should be completed by (date)

Name of EPA Contact Person

Telephone Number



## INSTRUCTIONS

Refer to 40 CFR 172 for regulations regarding experimental use permits. These regulations were published in the FEDERAL REGISTER on April 30, 1975 (40 FR 18780). Complete all (and only) numbered items on the application form. If an EPA Company Number (Item 2) has not previously been assigned, indicate "None," and a number will be assigned on your acknowledgment copy of the form. Third party applicants (those who will be testing another firm's registered product) need not complete Item 13. On the acknowledgment copy of this form, you will be assigned a File Number or Symbol for identification of this application. An expected completion date and the name of your EPA Contact will be entered. You may call your EPA Contact if you have not received your permit or a letter of explanation by the date indicated.

### Experimental Use Permit Data Submission

The following information must be submitted in triplicate and in detail (bound in removable sections A through G with margin tabs) for all new chemicals and many new products. For some new formulations, the information requested in Items C, D, E, and F may be included by reference to other formulations if adequate extrapolation may be made. Where the applicant requests permission to test a registered product, the information requested in Items B, E, F, and G below, along with the EPA Registration Number of the product, will usually suffice. Refer to 40 CFR 158.640 [53 FR 15993, May 4, 1988] for further information.

- A. A data sheet giving the chemical and physical properties of the chemical. A complete statement of the names and percentages by weight of each Active and Inert ingredient in the formulation to be shipped. This information will be handled as confidential material.
- B. One copy of the proposed label including directions for use necessary for evaluation of the product. Refer to 40 CFR 172.6 for minimum labeling requirements. In certain circumstances the experimental program or other supplemental labeling may be permissible in lieu of full labeling. In such cases, submit a full explanation as to how the labeling will be affixed to or accompany the container.
- C. Toxicity data or reference to available data on the toxicity of the pesticide including, where pertinent, data on the toxicity to fish and wildlife. Include a summary of this information. LD<sub>50</sub> values and results of eye irritation studies on the formulated product must be included.
- D. Residue data, where pertinent, on (a) food or feed commodities; (b) nonfood crops such as tobacco; and (c) foliage or other sites which may relate to worker hazard or adverse effects on the environment. Include a description of the analytical method(s) used and a summary of the data.
- E. Effectiveness data [required only if specified in Regulations 40 CFR 158.640, 53 FR 15993, May 4, 1988 and Registration Guidelines 40 CFR 158.202(i), 53 FR 15993, May 4, 1988].
- F. If the pesticide is to be tested in a manner involving food or feed, and an adequate tolerance is not established to cover the use, file a petition for a temporary tolerance with this Agency and forward three copies with this application. If appropriate tolerances are established already, cite applicable Regulation in Title 40 of the Code of Federal Regulations.
- G. Proposed Experimental Program:
  - (1) Give the qualifications and the names, addresses, and telephone numbers of the individuals (participants) who will supervise the experimental work.
  - (2) Name the States in which the pesticide will be used and the acreage to be treated in each State. Where "acreage" does not apply, give extent of testing per State in more appropriate terminology. Indicate separately any other State(s) to which the pesticide may be shipped for further distribution.
  - (3) Give the details of the proposed program including the types of target pests or organisms, the crops, animals, surfaces, materials, buildings, or sites of application to be treated and the major geographical areas where the material is to be used. For seasonal pests or crops, indicate the desired month for pesticide application to begin. Specify the use pattern, intended plot sizes, number of plots, number of replicates, dosage rates, methods of application, season of use (spring, summer, fall) and timing of application (preplant, postemergence, multiple (indicate pattern and number), etc.).
  - (4) List the objectives of the proposed program including, e.g., what type(s) of data will be collected during the testing period (performance, yield, phytotoxicity, environmental residue, etc.). Indicate your long-range testing plans, including how many years you expect to conduct experimental testing in support of registration of this use. This information will be helpful in evaluating the currently proposed program.
  - (5) Submit an explanation to justify the quantity of the material requested, including various parameters used to determine the quantity. Quantities authorized will be based on the program submitted and consideration of the types and amount of data required to support registration.
  - (6) Propose a suitable duration for the permit commensurate with the program. Any request for a period greater than 1 year must be adequately justified.
  - (7) State the method of disposition of any unused material left at the conclusion of the testing program.

### Paperwork Reduction Act Notice

The public reporting burden for this collection of information is estimated to average three quarters of an hour including time for reviewing instructions, gathering existing product sources and addresses, shippers to be used and addresses, and completing this instrument. Send comments regarding this estimate or any other aspect of this process, including suggestions for reducing the burden to: Chief, Information Policy Branch, PM-223, U.S. Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460; Office of Management and Budget, Paperwork Reduction Project (2070-0040), Washington, DC 20503.

NOTE: Applicant may retain last copy  
(04-14-93)



**MALLET 0.5G**

**FOR EXPERIMENTAL USE ONLY**

Experimental Use Permit Number:

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

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**Permittee:**

Kim Patten, Extension Specialist, Professor  
Washington State University Long Beach Research and Unit  
2907 Pioneer Road  
Long Beach WA 98631

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**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine ..... 0.5%

**OTHER INGREDIENTS:** ..... 99.5%

**TOTAL:** ..... 100.0%

**KEEP OUT OF REACH OF CHILDREN**

**CAUTION – CAUCION**

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No.

FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
NOTE TO PHYSICIAN	
No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS**  
**HAZARDS TO HUMANS AND DOMESTIC ANIMALS**  
**CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**  
**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
  - Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
  - Shoes plus socks
  - Protective eyewear when working in a non-ventilated space
- Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

PERSONAL PROTECTIVE EQUIPMENT (PPE)	
<b>Users must:</b>	
<ul style="list-style-type: none"> <li>• Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.</li> <li>• Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.</li> <li>• Remove PPE immediately after handling this product. Wash the outside of gloves before removing.</li> </ul>	

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.  
**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 1.0 lb a.i./ac using the following properly calibrated application equipment:

- conventional granular pesticide applicators ("Belly grinders")
- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~ 20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500ft.
- For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.
- Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between July 1 and October 31.
- A 100-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter (1/4) mile and all public boat launches within a one-and-a-half (1 1/2) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500-foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8 1/2 x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (1/4) of an inch high. Signs shall also state "Do Not Fish, Crab, or Clam". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

**SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any give wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.



### **Mixing and Loading Requirements**

The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

### **STORAGE AND DISPOSAL**

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Referr to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk thorough spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak insidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.





**ATTACHMENT 1 – Explanation and Justification**

Two indigenous species of burrowing shrimp severely impact both the mudflat community and oyster production in Willapa Bay and Grays Harbor, WA. Both ghost shrimp (*Neotrypaea californiensis*) and mud shrimp (*Upogebia pugettensis*) reside in burrows beneath the mudflat surface, where they abrogate habitat from other benthic organisms and severely disrupt the structure of the mudflat substrate by bioturbation, causing cultured and native bivalves to sink and die. Although indigenous, both species, but particularly ghost shrimp, have greatly increased in density and distribution in the last 60 years, likely due to a combination of factors including loss of seasonal freshwater influx since the damming of the Columbia River and a decrease in key predators due to over-fishing.

Since the 1960s, applications of carbaryl (Sevin® 80SP, Bayer Corp.) on selected and legally limited acreage of commercial oyster beds, have effectively suppressed burrowing shrimp. A single application usually sufficed through multiple years of oyster development. A suite of best management practices, such as seasonal placement of carbaryl to avoid migratory salmon and pre-season monitoring of target beds, ensured that the estuarine ecosystem was not significantly affected. However, the potential impact of many conventional (i.e., organophosphate and carbamate) pesticides has been questioned by a variety of groups. This was most recently demonstrated by the National Marine Fisheries Biological Opinion regarding the impact of three carbamate pesticides on Pacific Endangered Salmon. While the final outcome of that opinion has yet to be determined, it indicates an increasingly challenging future for the use of carbaryl against burrowing shrimp in Willapa Bay and Grays Harbor.

Without the ability to manage burrowing shrimp, a significant portion of the local shellfish industry would no longer be economically viable. In 1990, oyster aquaculture accounted for one of every twelve jobs in Pacific County. Since then, the decline in marine fisheries has made the local economy even more dependent on shellfish production. As demonstrated elsewhere, the collapse of agricultural and other resource-based industries often leads to increased private development and pollution.

Efforts by the Willapa Bay / Grays Harbor Oyster Growers Association (WGHOGA) to develop an IPM program have been ongoing since the inception of the carbaryl-based program, but were formalized in 2001 when a memorandum of agreement was signed with several organizations and state agencies to develop an IPM program. Investigations of alternatives to carbaryl currently involves dozens of scientists, extension agents, and grower-collaborators who focus on biological, mechanical, and chemical controls, as well as a better understanding of burrowing shrimp ecology. Some biological control options show potential for implementation in the future, but will require much more research. Some reduced risk compounds partially suppress burrowing shrimp populations, but densities remain above farmable levels. At this point, we have identified only a single alternative tactic, imidacloprid, that has sufficient efficacy, environmental compatibility, and potential for registration to control burrowing shrimp and allow shellfish farming to continue in Southwest Washington beyond 2012.



Although preliminary very small plot trials of imidacloprid (Admire 2EC @ 0.5 lb a.i./ac) showed efficacy comparable to carbaryl (Sevin WP or SP @ 10 lb a.i./ac), the results of last years commercial large scale trials were disappointing (see Effectiveness Data, Figure 6, Attachment 2). Hypothetical reasons for the general failure in efficacy suggested that a higher rate of the liquid formulation or the substitution of the liquid with a granular formulation of imidacloprid could be provide sufficient efficacy against burrowing shrimp at the commercial scale. Preliminary small plot trials this spring have supported that hypothesis (Effectiveness Data, Tables 23, 24).

So far, the maximum rate for imidacloprid on terrestrial crops has been 0.5 lb a.i./ac, as Terrestrial Field Dissipation Studies conducted by the original registrant (Bayer Corp.) were at that rate. However, the objective of those studies was to address transport of imidacloprid into ground water and from there into wells and the drinking water supply. The primary concern was to human health. Those trials were particularly critical to imidacloprid in those systems, where it is often applied as a seed coating against subterranean insect pests, thus its mode of entry into the ground water could theoretically be facilitated.

Our applications of imidacloprid to limited acreage in Willapa Bay will not leach into ground water, nor will it have any opportunity to enter drinking water reservoirs. Imidacloprid from our treatments will quickly dissipate into the hundreds of thousands of gallons of moving waters within the estuary. Furthermore, we wish to apply imidacloprid at a rate higher than 0.5 lb a.i./ac to only 35 of the total 67.5 acres for which we are applying (20 ac @ 2.0 lb a.i./ac and 15 ac @ 1 lb a.i./ac) (see Justification and Explanation of Quantity, Attachment 2). In addition, we plan to preliminarily examine the fate and transport of imidacloprid in association with the studies proposed here (Details of the Proposed Program, Attachment 2). Additional related studies include an anaerobic metabolism study, planned to initiate very soon, and a field sediment dissipation study, planned for next year's commercial trials.

We have initiated dialogue with the EPA, IR-4, and NuFarm to consider allowing a 3C registration by the WGHOA of liquid imidacloprid for this use at 2.0 lb a.i./ac and to understand what additional steps, if any, should be taken for such a registration. Both IR-4 and NuFarm support this approach.

These attachments and forms comprise the Application for an Experimental Use Permit to Ship and Use a Pesticide for Experimental Purposes Only (8570-17) with respect to imidacloprid to manage burrowing shrimp on Willapa Bay / Grays Harbor shellfish beds. The permit will allow us to continue tests of efficacy and non-target impact at a scale that more closely approximates commercial applications. These and subsequent tests will allow imidacloprid to advance toward registration and state permitting.



**ATTACHMENT 2****A) Chemical and Physical Properties**

- 1) Chemical names: 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine.
- 2) Molecular formula:  $C_9H_{10}ClN_5O_2$
- 3) Tradename: Imida E-AG 2 F (EPA Reg. No. 81959-22)
- 4) Formulation (2 lbs active ingredient per gallon) of imidacloprid
- 5) CAS Number: 13826-41-3
- 6) Molecular Weight: 255.7
- 7) Water Solubility: 0.51 g/l (200° C)
- 8) Solubility in Other Solvents: @ 20° C
  - a) dichloromethane - 50.0 - 100.0 g/l
  - b) isopropanol - 1.0-2.0 g/l
  - c) toluene - 0.5-1.0 g/l
  - d) n-hexane - <0.1 g/l
  - e) fat - 0.061 g/100g
- 9) Melting Point: 136.4-143.8° C., 143.8° C (crystal form 1) 136.4° C (crystal form 2)
- 10) Vapor Pressure: 0.2 uPa (20° C) ( $1.5 \times 10^{-9}$  mmHg)
- 11) Partition Coefficient: 0.57 (22° C). (Kidd, H. and James, D. R., Eds. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, 1991 (As Updated).10-2)
- 12) Adsorption Coefficient:
  - a) in a low organic carbon silt loam (0.9% OC),  $K_d = 2.4$  mL/g (Oi, M. 1999. Time-dependent sorption of imidacloprid in two different soils. J. Agric. Food Chem. 47: 327-332.13).
  - b) see Table 1. (Felsot and Rupert, 2002).

Table 1. Sediment Distribution Coefficients ( $K_d$ ) and Freundlich Sorption Coefficient ( $K_f$ ) for Imidacloprid in Willapa Bay Sediments and Sediments Mixed with Activated Carbon.

Initial solution concn, mg/L	sediment distribution coefficient ( $K_d$ , mgL/g)		
	CaCl <sub>2</sub>	saltwater	saltwater carbon/sediment (1:2)
0.01	0.59	0.52	3912
0.1	0.62	0.52	824
1	0.51	0.45	785
10	0.39	0.32	766
100	0.28	0.24	763
av $K_d$	0.48	0.41	1410
SD	0.14	0.13	1399
$K_f$	0.46	0.40	520
1/n	0.91	0.91	0.86

**B) Proposed Label**

See separate documents

**C) Toxicity Data and Summary** [1-7 mostly from ETOXNET (<http://extoxnet.orst.edu/pips/imidaclo.htm>)]

- 1) Acute toxicity
  - a) ORL-RAT: LD<sub>50</sub> 450 mg kg<sup>-1</sup> (Meister 1994)
  - b) ORL-MUS: LD<sub>50</sub> 131 mg kg<sup>-1</sup> (Kidd and James 1991)
  - c) 24-hour DML-RAT: >5,000 mg/kg.
  - d) Non-irritating to eyes and skin (rabbits), and non-sensitizing to skin (guinea pigs) (Kidd and James 1991)
- 2) Chronic Toxicity
  - a) A 2-year feeding study in rats fed up to 1,800 ppm resulted in a No Observable Effect Level (NOEL) of 100 ppm (5.7 mg/kg body weight in males and 7.6 mg/kg in females). Adverse effects included decreased body weight gain in females at 300 ppm, and increased thyroid lesions in males at 300 ppm and females at 900 ppm.
  - b) A 1-year feeding study in dogs fed up to 2,500 ppm resulted in a NOEL of 1,250 ppm (41 mg/kg). Adverse effects included increased cholesterol levels in the blood, and some stress to the liver (measured by elevated liver cytochrome p-450 levels) (Federal Register 1995).
- 3) Reproductive Effects
  - a) A three generation reproduction study in rats fed up to 700 ppm imidacloprid resulted in a NOEL of 100 ppm (equivalent to 8 mg/kg/day) based on decreased pup body weight observed at the 250 ppm dose level (Federal Register 1995).
- 4) Teratogenic Effects
  - a) A developmental toxicity study in rats given doses up to 100 ppm by gavage on days 6 to 16 of gestation resulted in a NOEL of 30 mg/kg/day (based on skeletal abnormalities observed at the next highest dose tested of 100 ppm) (Federal Register 1995)
  - b) In a developmental toxicity study with rabbits given doses of imidacloprid by gavage during days 6 through 19 of gestation, resulted in a NOEL of 24 mg/kg/day based on decreased body weight and skeletal abnormalities observed at 72 mg/kg/day (highest dose tested) (Pike et al. 1994).
- 5) Mutagenic Effects
  - a) Imidacloprid may be weakly mutagenic. In a battery of 23 laboratory mutagenicity assays, imidacloprid tested negative for mutagenic effects in all but two of the assays. It did test positive for causing changes in chromosomes in human lymphocytes, as well as testing positive for genotoxicity in Chinese hamster ovary cells (Pike et al. 1994).
- 6) Carcinogenic Effects
  - a) Imidacloprid is considered to be of minimal carcinogenic risk, and is thus categorized by EPA as a "Group E" carcinogen (evidence of noncarcinogenicity for humans). There were no carcinogenic effects in a 2-year carcinogenicity study in rats fed up to 1,800 ppm imidacloprid (Anatra-Cordone and Durkin 2005).
- 7) Organ Toxicity
  - a) In short-term feeding studies in rats, there were thyroid lesions associated with very high doses of imidacloprid (Pike et al. 1994).
- 8) Fate in Humans and Animals
  - a) Imidacloprid is quickly and almost completely absorbed from the gastrointestinal tract, and eliminated via urine and feces (70-80% and 20-30%, respectively, of the 96% of the parent compound administered within 48 hours). The most important metabolic steps include the degradation to 6-chloronicotinic acid, a compound that acts on the nervous system as described above. This compound may be conjugated with glycine and eliminated, or reduced to guanidine (USEPA 1995).



## 9) Toxicity to Aquatic Organisms

## a) Fish

## (1) Dose-response

- (a) bluegill (fresh): static 96-hr acute  $LC_{50}$  >105 mg a.i./L (Bowman and Bucksath 1990a)  
 (b) rainbow trout (fresh), chinook smolts (salt), sheepshead minnow (salt) (Table 2)  
 (c) chinook smolts (Figure 1)  
 (d) "Using the standard classification scheme proposed by U.S. EPA/EFED (2001), imidacloprid would be classified as practically nontoxic to fish."  
 (Anatra-Cordone and Durkin, 2005. Section 4.1.3.1, p 412)

Table 2. Toxicity of imidacloprid to fish (as presented in Anatra-Cordone and Durkin 2005, Appendix 5, except for †, C. Grue, unpublished data 2007)

Species	Exposure	Effects	Reference
<b>FRESHWATER Acute Toxicity:</b>			
Rainbow Trout ( <i>Ochorhynchus mykiss</i> ) mean length 5.3 cm, mean weight 1.3 g, 10 per concentration	Static 96-hour acute toxicity study with technical grade NTN 33893 (95.3% a.i.). Nominal concentrations of 0, 50, 89, 158, 281, 500 mg a.i./L, with measured greater than 80% of nominal values	48-hr $EC_{50}$ = 85 mg/L, 95% CI = 71 - 113 mg/L 48-hr NOAEC (immobility) = 42 mg/L Mobility was the endpoint of assessment	Young and Hicks 1990 MRID 42055317
Rainbow Trout † ( <i>Ochorhynchus mykiss</i> ) mean weight 0.3 g, 10 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Admire 2F (21.4% a.i.) Nominal concentrations of 0, 15, 22, 32, 46, 66, 96, 139, 202 mg a.i./L	96-hr $LC_{50}$ = 170 mg/L, 95% CI = 159 - 181 mg/L 96-hr NOAEC (lethargy) = 22 mg a.i./L (14% at 96 hr)	Grue and Frew unpublished data
Rainbow Trout † ( <i>Ochorhynchus mykiss</i> ) mean weight 23 g, 7 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Admire 2F (21.4% a.i.) Nominal concentrations of 0, 75, 107, 151, 215, 305 mg a.i./L	96-hr $LC_{50}$ = 163 mg/L, 95% CI = 148 - 177 mg/L 96-hr NOAEC (lethargy) = < 75 mg a.i./L	Grue and Frew unpublished data
White sturgeon † ( <i>Acipenser transmontanus</i> ) juvenile, mean weight 28 g 5 per concentration	Static 96-hour acute toxicity study with Nuprid 2F (21.4% a.i.) Nominal concentrations of 0, 46, 66, 96, 139, 202, 294 mg a.i./L measured concentrations at: T0 h: 50, 100, and 220 mg a.i./L for nominal of 46, 96 and 202 mg a.i./L; T96 h: 50, 100, and 220 mg a.i./L	96-hr $LC_{50}$ = 124 mg/L, 95% CI = 93 - 170 mg/L 96-hr NOAEC (lethargy) = 66 mg a.i./L (Figure 1)	Grue and Frew unpublished data
<b>FRESHWATER Chronic Toxicity:</b>			
Rainbow Trout ( <i>Ochorhynchus mykiss</i> ), newly fertilized eggs <4 hours old, 4 replicates of 35 eggs each per concentration, plus an additional 50 eggs per each of the 4 control replicates (egg viability determination)	98-Day flow-through early life stage test with technical grade NTN 33893 at nominal concentrations of 0, 1.3, 2.5, 5.0, 10 and 20 mg/L equivalent to mean measured concentrations of 0, 1.2, 2.3, 4.9, 9.8 and 19 mg/L	<u>original conclusions:</u> NOAEC = 9.8 mg/L LOAEC = 19 mg/L (statistically significant reduction in length at 36 and 60 days post-hatch, and body weight at 60 days posthatch). No statistically significant biologically important effects on egg viability, hatch, survival or behavioral variables were observed. MATC (maximum acceptable toxicant concentration) = 14 mg/L (geometric mean of NOAEC and LOAEC)	Cohle and Bucksath 1991 MRID 42055320

		<u>1992 re-evaluation:</u> Day 36 growth was most sensitive endpoint. Based on reevaluation of this endpoint: NOAEC = 1.2 mg a.i./L LOAEC = 2.3 mg a.i./L MATC = 1.7 mg a.i./L	Gagliano 1992 MRID 42466501
<b>SALTWATER Acute Toxicity:</b>			
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), young adult, mean length 29 mm, mean weight 0.77 g, 10 per concentration	Static 96-hour acute toxicity test of technical grade NTN 33893 (96.2% a.i.). Control, solvent control, 22.4, 35.2, 58.2, 105 and 195 mg/L mean measured concentrations	96-hour $LC_{50}$ = 161 mg a.i./L, 95% CI = 105 - infinity, NOAEC = 58.2 mg a.i./L on the basis of mortality and signs (lethargy, dark coloration) at higher concentrations.	Ward 1990a MRID 42055318
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), 4-day old, 10 per replicate, 4 replicates per concentration 24-h static renewal	Static 96-hour acute toxicity test of Imida EAG2F (21.4% a.i.) Nominal concentrations of 0, 10, 20, 40, 80, 160 mg a.i./L, mean measured concentrations to verify serial dilutions: 10, 78, and 150 mg a.i./L	96-hr $LC_{50}$ = 61 mg/L, 95% CI = 50-70 mg/L 96-hr NOAEC (lethargy) = 40 mg a.i./L	Frew, Grue and Curran, 2007 unpublished data
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), fertilized eggs, 15 per replicate, 4 replicates per concentration $\geq$ 80% hatch	32-day early life stage toxicity test (USEPA OPPTS 850.1400) of Imida E AG 2F (21.4% a.i.) Nominal concentrations of 0, 0.625, 1.25, 2.5, 5, and 10 mg a.i./L mean measured concentrations to verify serial dilutions: 0.59, 2.3, 9.5 mg a.i./L.	No adverse effects on survival or growth at any concentration tested. NOAEC = 10 mg a.i./L	Curran, Frew and Grue 2008, unpublished report, Nautilus Environmental
Chinook Salmon $\dagger$ ( <i>Ochorhynchus tshawtsha</i> ) mean weight 7 g, 10 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Imida 2F (21.4% a.i.) Nominal concentrations of 0, 46, 66, 96, 139, 202, 294 mg a.i./L	96-hr $LC_{50}$ = 109 mg/L (figure 2), 95% CI = 102 - 118 mg/L 96-hr NOAEC (lethargy) = 66 mg a.i./L (Figure 2)	Grue and Frew unpublished data

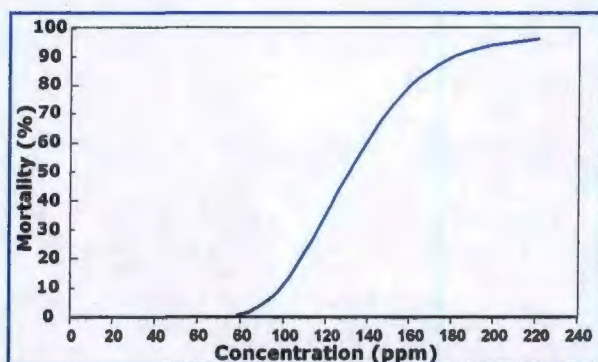


Figure 1 Dose-response curve for White sturgeon juveniles exposed to Nuprid 2F in freshwater for 96 hr.  $LC_{50}$  = 124 mg a.i./L, CI = 93 - 170 mg a.i./L. C. Grue unpublished data

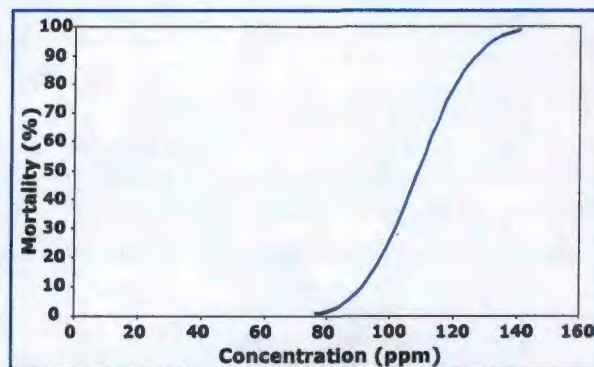


Figure 2 Dose-response curve for Chinook smolts (7g) exposed to Imida 2F in seawater for 96 hr.  $LC_{50}$  = 109 mg a.i./L, CI = 102 - 118 mg a.i./L. C. Grue, unpublished data



(2) Local (Willapa) Field Tests (Table 3; Patten et al., 2007)

(3) Local (Willapa) Lab Tests (Table 4; Patten et al., 2008)

Saddleback gunnel collected in Willapa Bay and maintained in aquaria for 5 days prior to testing. 5 fish per replicate, 3 replicates per concentration. Fish exposed to imidacloprid in estuarine water (56 – 64° F) in 1 L jars.

Table 3. Effects of carbaryl (Sevin) and imidacloprid (Imida) overspray on fish in tide pools.		
Treatment	% survival at 48 hr after treatment	
	staghorn sculpin	threespine stickleback
Sevin 80SP (8 lb a.i./ac)	11.3 <i>b</i>	64.0 <i>b</i>
Imida 2F (0.5 lb a.i./ac)	100.0	100.0
untreated check	100.0	100.0
* means followed by the same letter are not significantly different (Duncans Multiple Range; P=0.05).		

Table 4. Effects of imidacloprid concentration and exposure time on survival of saddleback gunnel ( <i>Pholis ornata</i> ).				
Concentration (ppm)	% Survival			
	4 hr	24 hr	48	96 hr
0	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>
10	100.0	100.0	100.0	100.0
100	100.0	100.0	100.0	93.3
* means followed by the same letter are not significantly different (LSD; P=0.05). <i>n.s.</i> , not significant				

## b) Relevant Aquatic Invertebrates (Freshwater Insects not included)

## (1) Dose Response Parameter (Table 5).

From Anatra-Cordone and Durkin, 2005: "Amphipod crustaceans such as *Hyaella azteca*, the saltwater Mysid, *Mysidopsis bahia*, and the fresh water insect midge, *Chironomus tentans*, are the most sensitive species. In freshwater, the water flea, *Daphnia magna*, was the least sensitive species, while in saltwater, the eastern oyster is least sensitive. Acute toxicity values range from a 96-hour NOAEC of 0.000035 mg/L for *H. azteca* (England and Bucksath 1991), to a 96-hour NOAEC of 145 mg/L for eastern oyster (Wheat and Ward 1991). On the basis of longer-term studies designed to assess reproduction, growth and survival, *M. bahia* was the most sensitive species, with an NOAEC value of 0.000163 mg a.i. imidacloprid/L for growth and reproductive success (Ward 1991), and *D. magna* was the most tolerant species with a 21-day NOAEC for immobility of 1.8 mg/L (Young and Blake 1990)."

Table 5. Toxicity of imidacloprid to relevant aquatic invertebrates (mostly as presented in Anatra-Cordone and Durkin, 2005; Appendix 6).			
Species	Exposure	Effects	Reference
<b>FRESHWATER Acute Toxicity:</b>			
Water flea ( <i>Daphnia magna</i> ), 2 flasks per concentration with 10 each	Static 48-hour acute toxicity study with NTN 33893 (95.9% a.i.) at nominal concentrations up to 125 mg/L with actual mean concentrations of 0, 15, 25, 42, 71 and 113 mg/L	96-hour LC50: 211 mg a.i./L (158 - 281 mg a.i./L). 96-hour NOAEC: 50 mg a.i./L 89 mg/L and higher: apathy, irregular swimming behavior, lying on side/back, staggering 281 mg/L and higher: mortality	Grau 1988a MRID 42055316 Ward 1990a MRID 42055318
<i>Hyaella azteca</i> (amphipod crustacean), 2-3 mm juveniles, 2 replicates per concentration, 10 per replicate	Static acute toxicity test with NTN 33893 at measured concentrations of control, 0.00035, 0.00097, 0.0035, 0.010, 0.034, 0.100, 0.340, 1.000 and 3.100 mg/L	96-hr LC50: 0.526 mg/L, 95% CI = 0.194 - 1.263 mg/L 96-hr EC50 (immobilization): 0.055 mg/L, 95% CI = 0.034 - 0.093 mg/L 96-hr NOAEC (immobilization and abnormal effects, such as lethargy or surfacing) = 0.00035 mg/L	England and Bucksath 1991 MRID 42256303



<i>Hyalella azteca</i> (amphipod crustacean), 14 - 21 days old, 2 replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33823 metabolite at mean measured concentrations of 0, 5.6, 11.0, 22.1, 43.8 and 86.8 mg/L	96-hour LC50: 51.8 mg a.i./L, 95% CI = 44.0 - 60.9 mg a.i./L 96-hour EC50 (immobilization): 29.0 mg a.i./L, 95% CI = 24.7 - 34.0 mg a.i./L 96-hour NOAEC (mortality): 22.1 mg a.i./L	Rooney and Bowers 1996 MRID 43946601
<i>Hyalella azteca</i> (amphipod crustacean), 7 - 21 days old, 2 replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33519 urea metabolite at nominal (measured) concentrations of 0, 6.25 (5.81), 12.5 (11.80), 25 (23.46), 50 (46.80), and 100 (94.83) mg a.i./L	96-hour LC50: > 94.83 mg a.i./L, 96-hour EC50 (immobilization): > 94.83 mg a.i./L, 96-hour NOAEC: 94.83 mg a.i./L	Dobbs and Frank 1996a MRID 43946603
<b>FRESHWATER Chronic Toxicity:</b>			
Water flea ( <i>Daphnia magna</i> ), 4 replicate jars per concentration, 6 1 <sup>st</sup> instar daphnids per jar	Chronic static renewal toxicity study of technical grade NTN 33893. Control, solvent control, 0.46, 0.86, 1.8, 3.6, and 7.3 mg/L	21-day EC50 (imobilization): >7.3 mg/L MATC = 2.5 mg/L (1.8 - 3.6 mg/L) NOAEC = 1.8 mg/L LOAEC = 3.6 mg/L 3.6 and 7.3 mg/L: Significantly reduced adult daphnid length in comparison with pooled controls 7.3 mg/L: Significantly reduced survival; significantly reduced mean young/adult reproduction days in comparison with pooled controls. No effects on time to first brood at any concentration.	Young and Blake 1990 MRID 42055321
<b>SALTWATER Acute Toxicity:</b>			
<i>Artemia</i> sp., and Mosquito ( <i>Aedes taeniorhynchus</i> ) 3 trials, 4 replicates per concentration, 10 animals each species per replicate	Static 48-hr acute toxicity test. Technical grade imidacloprid (>95% purity)	<u>Artemia:</u> 48-hr LC50 = 361.23 mg/L, 95% CI = 307.83 - 498.09 mg/L <u>Mosquito:</u> 48-hr LC50 = 0.13 mg/L, 95% CI = 0.010 - 0.016 mg/L Note: increasing salinity increased sensitivity to imidacloprid	Song et al 1997; Song and Brown 1998
Mysid ( <i>Mysidopsis bahia</i> ), < 24 hours old, 10 per concentration.	96-hr flow-through acute toxicity tests of technical grade NTN 33893 (96.2% a.i.). Mean measured concentrations: 1 <sup>st</sup> test: control, solvent control, 0.032, 0.0584, 0.0937, 0.146 and 0.249 mg a.i./L 2 <sup>nd</sup> test: control, solvent control, 0.00842, 0.0133, 0.0229, 0.0372 and 0.0634 mg a.i./L	<u>First test:</u> 96-hr LC50 = 0.0377 mg a.i./L, 95% CI = 0.0267 - 0.0464 mg a.i./L, NOAEC not determined. <u>Second test:</u> 96-hr LC50 = 0.0341 mg a.i./L, 95% CI = 0.0229 - 0.0372 mg a.i./L, NOAEC = 0.0133 mg a.i./L on the basis of mortality and loss of equilibrium at higher doses.	Ward 1990b MRID 42055319
Mysid ( <i>Mysidopsis bahia</i> ), < 24 hours old, 2 replicates per concentration, 10 per replicate	96-Hr flow-through acute toxicity test, NTN 33893 240 FS Formulation, control, solvent control, 18 (21), 29 (31), 49 (56), 82 (78), 136 (125) and 227 (219) ug a.i./L nominal (measured) concentrations	96-hr LC50 = 0.036 mg a.i./L, 95% CI = 0.031 - 0.042 mg a.i./L NOAEC (mortality) = 0.021 mg a.i./L	Lintott 1992 MRID 42528301



Eastern Oyster ( <i>Crassostrea virginica</i> ), 20 per concentration	96-hr flow-through test of effect on shell growth. Technical grade NTN 33893 (95.8% and 96.2% a.i. for 2 <sup>nd</sup> and 1 <sup>st</sup> tests, respectively) 1 <sup>st</sup> test: control, solvent control, 2.93, 5.14, 8.19, 14.2, and 23.3 mg a.i./L, measured 2 <sup>nd</sup> test: control, 145.0 mg a.i./L, measured	<u>First test:</u> 100% survival; No effects on new shell growth <u>Second test:</u> 100% survival; new shell growth of exposed was 22% less than controls. This was statistically significant. 96-hr NOAEC: 145 mg/L	Wheat and Ward 1991 MRID 42256305
<b>SALTWATER Chronic Toxicity:</b>			
Midge ( <i>Chironomus tentans</i> ), second instar, 2 replicates per concentration, 10 chironomids per replicate	Static renewal 96-hr toxicity test with technical grade NTN 33893 (95.0 % a.i.) control, solvent control, measured concentrations of 0.00067, 0.00124, 0.00339, 0.0102, 0.0345, 0.100, and 0.329 mg a.i./L	10-day LC50: 0.00317 mg/L, 95% CI = 0.00124 - 0.0102 mg/L 10-day survival NOAEC: 0.00124 mg/L 10-day growth NOAEC: 0.00067 mg/L (basis = dry weight of survivors)	Gagliano 1991 MRID 42256304
Mysid ( <i>Mysidopsis bahia</i> ), <24- hrs old, 4 replicates per concentration, 15 mysids per replicate cup	Flow-through chronic toxicity tests with technical grade NTN 33893 (96.2% a.i.) <u>First test:</u> control, solvent control, 560, 1290, 2850, 5080 and 10100 ng a.i./L mean measured <u>Second test:</u> control, solvent control, 36.8, 78.4, 163, 326 and 643 ng a.i./L nominal	<u>First Test:</u> <u>1290 ng/L and higher:</u> Significantly reduced number of offspring per female reproductive day <u>5080 ng/L and higher:</u> significantly reduced growth of 1 <sup>st</sup> generation mysids as total length and dry weight <u>10,100 ng/L:</u> Statistically increased mortality in comparison with pooled controls for first generation. No effects on mortality in 2 <sup>nd</sup> generation <u>MATC (reproductive success):</u> 849 ng/L (560 - 1290 ng/L) <u>MATC (growth):</u> 3806 ng/L (2850-5080 ng/L) <u>Second Test:</u> No effects on number of offspring per female reproductive day. <u>326 and 643 ng/L:</u> Significantly reduced growth of 1 <sup>st</sup> generation as total length and dry weight in comparison with pooled controls <u>643 ng/L:</u> Statistically increased mortality in comparison with pooled controls for 1 <sup>st</sup> generation. No effects on mortality in 2 <sup>nd</sup> generation. <u>MATC (reproductive success):</u> > 643 ng/L <u>MATC (growth):</u> 230 ng/L (163 - 3260 ng/L) No real explanation for discrepancy between 1 <sup>st</sup> and 2 <sup>nd</sup> tests with regard to growth.	Ward, 1991 MRID 42055322

(2) Local (Willapa) Tests  
(Patten, unpublished data)

i) Diploid oyster larvae  
(a) Survival (Table 5)

All tests featured diploid Pacific oyster larvae from  
Taylor Shellfish within 2 weeks of test. No of  
individuals per replicate and type of arena as

Table 5. Effects of imidacloprid on survival of diploid  
Pacific oyster larvae following 24 hr exposure in 3 arenas.

Arena	Sample Size	Concentration (ppm)	% Survival *
test-tube	15 - 20	0	67.2 n.s.
		1	69.7
		5	47.1
		10	30.7
		20	41.6



specified. 3 replicates per concentration. Tests in water bath at 79 – 80°F for 24 hr. Oysters identified as live or dead based on swimming activity.

Percent survival was not significantly different from plain estuarine water at less than 50 ppm imidacloprid. (Patten unpublished data, 2008)

(b) Survival set, growth (Table 6)

As above, except 4 replicates per concentration; 3 oyster shells per 1 L glass jar. Survival measured after 24 hr exposure and shells transferred to growout bags in Willapa Bay, 6 inches above the tidal substrate, at -1.0 tide height. Number of set oysters and diameter measured after 158 days growout.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

ii) Set, growth of triploid oyster larvae (Table 7)

As above, except triploid Pacific oyster larvae obtained from Taylor Shellfish within 2 weeks of testing, 4 shells per replicate / jar, diameter measured after 172 days in growout bags after 24 hr exposure to imidacloprid.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

iii) Growth of diploid Pacific juvenile oysters (Table 8)

As above, except 5 small juvenile ( $\times$  surface area = 8.5 mm<sup>2</sup>) diploid Pacific oysters per shell, 3 shells per replicate, 3 replicates per concentration, exposed to imidacloprid in fresh estuarine water for 96 hr, then transferred to growout bags for 158 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

iv) Growth of diploid juvenile oysters (Table 9)

As above, except initial juvenile diploid Pacific oyster length was 7.8 mm, 6 oysters per replicate, 3 replicates per treatment, growout for 273 days.

Impact was not significantly different from estuarine water at any concentration or variable (Patten unpublished data, 2008)

250 ml cups	30 – 40	0	15.7 <i>b</i>
		1	10.0 <i>b</i>
		10	18.0 <i>b</i>
		100	0 <i>a</i>
1 L jars	10 – 25	0	48.0 <i>n.s.</i>
		1	28.0
		10	69.0
		20	23.0
1 L jars	30 – 70	0	38.0 <i>b</i>
		5	6.0 <i>b</i>
		50	0 <i>a</i>
		500	0 <i>a</i>

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 6. Effects of imidacloprid on survival, set, and development (diameter) of diploid Pacific oyster larvae after 24 hr exposure.

Sample Size	Concentration (ppm)	% Survival*	No. Set	Diameter (mm)
100 – 150	0	54.5 <i>n.s.</i>	9.3 <i>n.s.</i>	7.8 <i>n.s.</i>
	10	42.0	15.8	8.8
	100	33.0	14.8	8.7
	1000	42.7	18.0	8.6

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 7. Effects of imidacloprid on set and development (diameter) of triploid Pacific oyster larvae following 96 hr exposure.

Sample Size	Concentration (ppm)	No. Set	Diameter (mm)
14 – 150	0	2.4 <i>n.s.</i>	21.9 <i>n.s.</i>
	5	1.3	26.3
	50	1.1	28.1

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 8. Effects of 96 hr exposure to imidacloprid on development of diploid juvenile oysters after 158 days growout.

Concentration (ppm)	Surface Area (mm <sup>2</sup> )
0	8639 <i>n.s.</i>
10	10071
100	9306
1000	7797

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 9. Effects of imidacloprid at 48 and 96 hr exposures on length of juvenile (7.8 mm length) oysters after 273 days growout.

Concentration (ppm)	Length (mm)	
	48	96
0	54 <i>n.s.</i>	48 <i>n.s.</i>
10	53	42
100	37	46
1000	59	39

\* means followed by the same letter are not significantly different (LSD; P=0.05).



v) Growth of juvenile Kumomoto oysters  
(Table 10)

As above, except 5 small juvenile ( $\bar{x}$  diameter = 18 mm<sup>2</sup>)

Kumomoto oysters from Taylor Shellfish per replicate, 3 replicates per concentration, exposed to imidacloprid in fresh estuarine water for 48 or 96 hr, then transferred to growout bags for 92 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

Table 10. Effects of imidacloprid on development (diameter) of juvenile Kumomoto oysters after 24 or 96 hr exposure and 92 days growout.

Concentration (ppm)	Diameter (mm <sup>2</sup> )	
	24 hr	96 hr
0	28.2 <i>n.s.</i>	27.9 <i>n.s.</i>
10	23.4	26.3
100	25.5	27.3

means followed by the same letter are not significantly different (LSD; P=0.05).

vi) Manila clams

(a) Preliminary tests by size  
(Figure 3)

Water temperatures for 3 – 6 mm clams, 67° F, others, 48 – 49°F. Survival rates were > 50% for all size classes at imidacloprid concentrations < 1000 ppm (Patten, unpublished data, 2007)

(b) Small clams, (Table 11)

Methods as above for 2008 lab tests, except ~120 small ( $\bar{x}$  diameter = 4.75 mm) Manila clams per replicate / 1 L jar, 5 replicates per concentration. Clams were triple rinsed after treatment then placed on sieved sand. Mortality assessed as not burrowing in sand after 24 hr. Live clams placed in 1 mm mesh growout bags for 30 days, then transferred to 2 mm mesh bags for 46 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

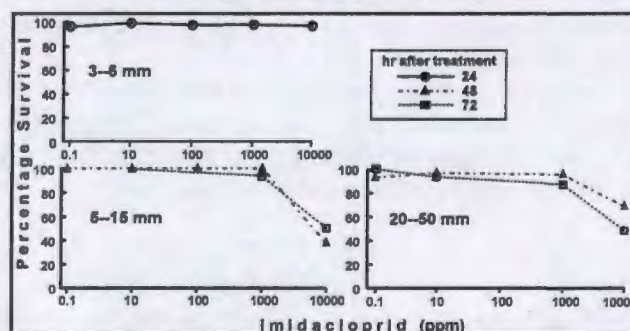


Figure 3 Effects of imidacloprid (Admire 4.6F) on Manila clams of different size classes.

Table 11. Effects of imidacloprid on survival of juvenile Manila clams at exposure intervals and development (diameter) after 76 days growout.

Exposure Interval	Concentration (ppm)	% Survival	Diameter (mm)
48	0	91.7 <i>n.s.</i>	6.0 <i>n.s.</i>
	1	94.5	6.4
	10	90.8	6.9
	100	87.8	5.4
96	0	93.3 <i>n.s.</i>	6.8 <i>n.s.</i>
	1	92.5	7.7
	10	90.2	7.0
	100	91.1	5.9

\* means followed by the same letter are not significantly different (LSD; P=0.05).

vii) Dungeness crab megalopae

(a) Preliminary 2008 trials.

Collected as megalopae using light trap on June 16, 2008, but most metamorphosed to first post-larval instar during exposure to imidacloprid 7 days later. Single individual per replicate, 3 replicates per concentration, 3 exposure intervals per concentration. No mortality at any treatment combination of 0, 10, 100 ppm imidacloprid and 4, 24, 48, and 96 hr exposure intervals.

viii) Juvenile Dungeness Crab

(a) Initial 2007 trials

Mortality was very low in juvenile crab (carapace width < 3") exposed to 0.5 lb a.i./ac imidacloprid in the field (Table 12; Patten unpublished data), but larger crab showed substantial tetanus shock in large scale field trials (see below).

Table 12. Two tests of carbaryl (Sevin 80SP) and imidacloprid (Imida 2F) overspray on juvenile Dungeness crab in tide pools.

Treatment	Days After Treatment	% Mortality*
Sevin 80SP (8 lb	14	70 <i>b</i>
Imida 2F (0.5 lb a.i./ac)	14	0.208333333
untreated check	14	0
Imida 2F (5.0 lb a.i./ac)	21	90 <i>a</i>
untreated check	21	86 <i>a</i>

\* means followed by the same letter are not significantly different (Duncans Multiple Range; P=0.05).



## (b) 2009 trials

Crab were collected as megalopae over three nights in late May and maintained in aerated seawater until testing on May 27. 10 megalopae were placed as a replicate in a 10 ml container containing each of 4 imidacloprid concentrations (0.5, 1, 5, and 10 ppm). Four replicates were exposed to each concentration for 4 hr, after which 5 megalopae per rep were removed rinsed in seawater, and placed in individual 1 L aerated jars. Remaining megalopae were exposed for another 14 hr, and then similarly rinsed and placed in jars. Megalopae were observed for tetanus and mortality at 35 and 131 hr after initial treatment.

Although large percentage of the test populations were in shock at 35 hr after exposure, especially at the higher rate and longer exposure interval, survival was equally high or even greater (Table 13).

ix) Benthic Infauna (Figure 3; Booth unpublished data, 2007).

Absolute abundance of non-target invertebrates was significantly lower in plots treated with imidacloprid (Admire 1.6F; 0.4 lb a.i./ac) compared to plots treated with carbaryl (Sevin 80SP; 1 lb a.i./ac) or left untreated.

Neither Species Richness nor Simpson's Diversity differed significantly among treatment plots at both short and long post-treatment intervals.

Table 13. Effects of imidacloprid at to induce tetanus shock (measured at 35 hr post treatment) and on survival (at 131 hr post treatment) of crab megalopae.

Exposure Interval (hr)	Concentration (ppm)	% in Shock	% Survival
4	0.5	45	85
	1	55	95
	5	75	80
	10	95	65
18	0.5	100	85
	1	90	85
	5	100	80
	10	100	100

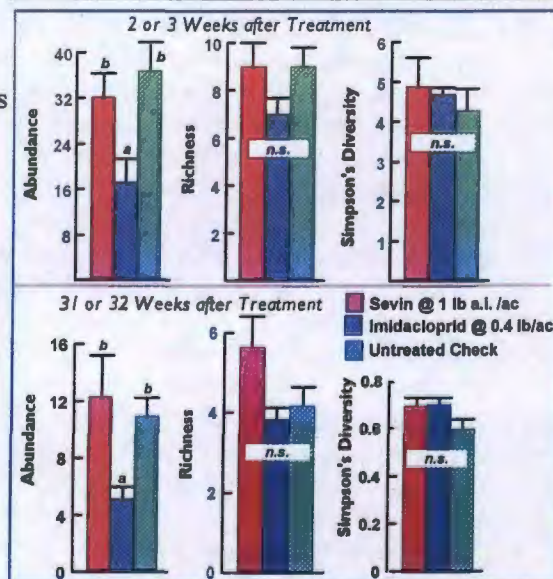


Figure 4 Affects of carbaryl (Sevin 80S) and imidacloprid (Admire 4.6F) on non-target benthic invertebrates at short and long post-treatment intervals.

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#### D) Residue Data

##### 1) Food

- a) In general: an examination of the USDA PDP (Pesticide Data Program) database for FY2004 and FY2005 showed that only about 25% of food samples had detectable imidacloprid residues. Considering that the acute dietary risk assessment scenario assumed that all imidacloprid commodity residues were at tolerance levels and 100% of all crops were treated, the actual acute dietary exposure would be significantly lower than assessed for the Registration Eligibility Decision (Cutchin 2007).
- b) For fish taken for recreation or subsistence consumption under this proposed EUP and associated program: significant exposures to imidacloprid are unlikely given the limited acreage requested and in light of the rapid dissipation of residues following bed treatment (Felsot and Ruppert 2002).
- c) For shellfish: because most beds will be treated with a planted crop of seed which take multiple years of development prior to harvest, the likelihood of any imidacloprid residues remaining unmetabolized is extremely low, especially in light of its  $K_{ow}$ , as explained in Section F. (Petition for Temporary Tolerance) below.
- d) For oysters: using the fugacity based FISH model and appropriate assumptions, estimates of residues in fish (and hypothetically oysters) ranged on a whole body basis from 0.814  $\mu\text{g/kg}$  to 21.1  $\mu\text{g/kg}$  (the assumed body tissue density was 1  $\text{kg/L}$ ). A detailed explanation for the derivation of these concentrations, as well as exposure estimates, are presented in Section F. (Petition for Temporary Tolerance) below.

##### 2) Worker Safety

- a) Exposure estimates for aerial applicators to forest canopy has been calculated at 0.005  $\text{mg/kg/day}$  (Anatra-Cordone, M. and P. Durkin. 2005. Imidacloprid - Human Health and Ecological Risk Assessment – Final Report. Prepared for USDA, Forest Service, Forest Health Protection, GSA Contract No. 10F-0082K, USDA Forest Service BPA: WO-01-3187-0150, USDA Purchase Order No.: 43-1387-4-3131, Task No. 24. Submitted by Syracuse Environmental Research Associates, Inc., 5100 Highbridge St., 42C, Fayetteville, New York 13066-0950)
- b) The re-entry interval (REI) to commercial oyster and clam beds will likely be the same as the labeled REI for other imidacloprid products (e.g., Admire, Guacho) of 12 hours. The 12 hour restriction has limited relevance, as shellfish workers generally have no need to enter sprayed plots for several days, if not weeks, following application. Shellfish beds sprayed at low tides will also be submerged within 12 hours by the intervening high tides, substantially diluting imidacloprid concentrations in water and on substrate.



**E) Effectiveness Data****1) Small plot trials, 2006 – 2008**

Imidacloprid (Admire 1.6F, Bayer Corp.; Imida 2F, Etigra) has been tested for efficacy against burrowing shrimp since 2006 in several small plot (e.g., 3m<sup>2</sup>, 10m<sup>2</sup>, 10×20m, or 3×20m) trials, as Washington State EUP acreage limit is 0.1 ac per year. Imidacloprid was sometimes applied along with other compounds (e.g., flowable sulfur, pyrethrins, and pyrethroids), but was most often compared to carbaryl applied at a lower than standard rate (e.g., 3 vs 8 lb a.i./ac) and an untreated check. In initial (2006) broadcast trials, imidacloprid was effective at a range of rates and at a long post treatment interval (Table 14).

Table 14. Affects of carbaryl (Sevin 80WP), 5 rates of imidacloprid (Admire 1.6F) and an untreated check on # burrows/m<sup>2</sup> ( $\bar{x} \pm SE$ ) at 45 and 255 days after treatment (DAT), 2006.

Pesticide	Rate (lb a.i./ac)	Burrow Density*	
		45 DAT*	255 DAT
Sevin	3	16.0 $\pm$ 5.5 a,b	17.3 $\pm$ 3.8 a
Admire	0.05	29.7 $\pm$ 9.4 b	38.0 $\pm$ 6.0 b
	1	15.7 $\pm$ 7.1 a,b	18.0 $\pm$ 9.1 a
	2	1.7 $\pm$ 0.9 a	2.0 $\pm$ 1.0 a
	3	1.0 $\pm$ 0 a	0 a
	4	0 b	0
Untreated	0	73.7 $\pm$ 4.9 c	69.7 $\pm$ 6.9 c

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Our research also included the potential of subsurface injection technologies. In 2004 – 2005, we assessed nozzle and spikewheel injection of non-imidacloprid compounds from semi-amphibious vehicles at low tide. In 2006, a 6' wide apparatus holding 4 spikewheels was mounted on a pontoon raft which was pushed over plots with a boat. Imidacloprid was tested multiple times at various rates and locations using the underwater spikewheel technology. Usually, efficacy of imidacloprid was greater (post treatment burrow density was lower) at higher rates, but the response was not always linear. At a test area near Nahcotta, where substrates were primarily sandy, burrow densities were substantially, if not significantly, higher at rates less than 0.2 lb a.i./ac. This was especially true at longer post application intervals (e.g., 42 or 50 days after treatment) (Table 15, Trials 1, 2). Efficacy was not always greater in plots treated with imidacloprid at rates greater than 0.2 lb a.i./ac (Table 15, Trial 2: 2<sup>nd</sup> and 3<sup>rd</sup> post application interval; Trial 5). Burrow density was also significantly lower in plots treated with 2.0 lb a.i./ac imidacloprid than in plots treated with 3.0 lb a.i./ac carbaryl (Table 15, Trial 1).

Table 15. Affects of carbaryl (Sevin 80SP) and imidacloprid (Admire 4.6F), injected subsurface using underwater spikewheels, on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 5 trials and up to 3 post application intervals (PAI, days after treatment (DAT)) in a sandy substrate at Nahcotta. 2006.

Trial	Treatment	Rate (lb)	Burrow Density*		
			1 <sup>st</sup> PAI†	2 <sup>nd</sup> PAI‡	3 <sup>rd</sup> PAI§
1	Sevin	3	14.7 $\pm$ 3.1 b,c	28.6 $\pm$ 2.9 b	16.4 $\pm$ 1.0 b
		0.05	23.2 $\pm$ 8.1 c	43.6 $\pm$ 2.9 b	NA
	Admire	0.1	5.7 $\pm$ 2.5 a,b	33.1 $\pm$ 2.7 a	NA
		0.2	0.25 $\pm$ 0.2 a	18.2 $\pm$ 1.9 a	13.6 $\pm$ 1.0 a
		Untreated	0	81.0 $\pm$ 2.1 d	91.7 $\pm$ 1.5 c
2	Admire	0.124	23.3 $\pm$ 11.8 a	47.3 $\pm$ 1.6 b	32.4 $\pm$ 1.5 b
		0.25	0.7 $\pm$ 1.2 a	24.9 $\pm$ 3.6 a	17.9 $\pm$ 2.1 a
		0.5	0	22.0 $\pm$ 4.3 a	16.2 $\pm$ 1.9 a
	Untreated	0	62.0 $\pm$ 9.5 b	91.7 $\pm$ 1.5 c	NA
		Untreated	0	91.7 $\pm$ 1.5 c	NA
3	Admire	0.2	0.2 $\pm$ 0.2 a	0.7 $\pm$ 0.4 a	NA
	Untreated	0	81.0 $\pm$ 2.1 b	95.3 $\pm$ 3.1 b	NA
		Untreated	0	95.3 $\pm$ 3.1 b	NA
4	Admire	0.1	12.2 $\pm$ 2.7 b	NA	NA
		0.2	2.4 $\pm$ 0.7 a	NA	NA
	Untreated	0	72.4 $\pm$ 3.8 c	NA	NA
		Untreated	0	NA	NA
5	Admire	0.2	6.5 $\pm$ 1.6 a	NA	NA
	Untreated	0	105.4 $\pm$ 4.7 b	NA	NA

\* means followed by the same letter are not significantly different (LSD or t-test; P=0.05).

† Trial 1, 14 DAT; Trial 2, 6 DAT; Trial 3, 10 DAT; Trial 4, 14 DAT.

‡ Trial 1, 42 DAT; Trial 2, 50 DAT; Trial 3, 21 DAT; Trial 4, 21 DAT.

§ Trial 1, 249 DAT; Trial 2, 258 DAT.



Results of a trial conducted on sandy/silty substrates were confounded somewhat by heavy growths of eel grass (primarily invasive *Zostera japonica*, but also *Z. marina*), which slowed tidal drainage, left standing water on the bed, and obscured burrow counts (Table 16).

Another trial, conducted at the Willapa Bay Fish and Wildlife Refuge, featured applications of imidacloprid (Admire 2F; 0.2 lb a.i./ac) on four different types of substrate. Burrows were counted in four 1 m<sup>2</sup> quadrants within and in a single 1 m<sup>2</sup> plot adjacent to each treatment plot. Shrimp burrow density was significantly lower in all treated compared to untreated plots ( $\bar{x} \pm SE$ , 52.2  $\pm$  15.7 burrows/m<sup>2</sup>; LSD, P=0.05), but was significantly higher in a plot of silty hummocks than in plots of other substrate types (Table 17).

In 2007, three broadcast trials continued to demonstrate the fast action and fairly long-lasting efficacy of imidacloprid on burrow density (Table 18).

Table 17. Affects of imidacloprid (Admire 1.6F) at 0.2 lb ai/ac on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) on different substrate types at 13 days after treatment, 2006.

Treatment	Substrate	Burrow Density*
Admire	Oyster Shell	2.8 $\pm$ 0.6 a
	Silt	3.2 $\pm$ 3.2 a
	Sand / Silt	8.8 $\pm$ 4.3 a
	Silt Hummocks	19.0 $\pm$ 0.6 a

\* means followed by the same letter are not significantly different (LSD; P=0.05).  
Untreated check (52.2  $\pm$  15.7) not included in analysis

Other small plot trials conducted in 2007 and 2008 examined the efficacy of imidacloprid when spikewheel injected by boat or ATV, sediment type, and eelgrass cover on the efficacy of imidacloprid (Table 19). None of the sites featuring application by spikewheel showed outstanding control, whereas burrow density was reduced by  $\geq 95\%$  compared to burrow density in untreated plots when application was by broadcast.

Table 16. Affects of imidacloprid (Admire 1.6F) on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) at 10 days after treatment in sand / silt at Middle Island Sands.

Treatment	Rate (lb a.i./ac)	Burrow Density*
Admire	0.2	4.2 $\pm$ 2.0 a
	0.4	8.1 $\pm$ 1.7 a
Untreated	0	33.5 $\pm$ 2.6 b

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 18. Affects of imidacloprid (Imida 2.F) on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 3 trials and at 2 post application intervals (PAI, days after treatment (DAT)) at Nahcotta, 2007.

Trial	Treatment	Rate (lb a.i./ac)	Burrow Density*	
			1 <sup>st</sup> PAI †	2 <sup>nd</sup> PAI ‡
1	Imida	0.5	0	0
		0.25	0.2 $\pm$ 0.1 a	1.8 $\pm$ 0.9 b
		0.125	2.9 $\pm$ 1.1 a	18.3 $\pm$ 4.5 b
	Untreated	0	119.5 $\pm$ 2.4	71.7 $\pm$ 2.4 c
2	Imida	0.5	0	1.3 $\pm$ 0.7 a
		0.25	6.3 $\pm$ 3.1 b	15.0 $\pm$ 4.7
		0	26.1 $\pm$ 4.8	71.7 $\pm$ 2.4
	Untreated	0	85.6 $\pm$ 3.9	94.7 $\pm$ 5.2 c
3	Imida	0.5	7.5 $\pm$ 1.6 a	5.8 $\pm$ 2.5 a
		0.25	16.2 $\pm$ 2.3	48.9 $\pm$ 6.2 b
		0	85.6 $\pm$ 3.9	94.7 $\pm$ 5.2 c
	Untreated	0	85.6 $\pm$ 3.9	94.7 $\pm$ 5.2 c

\* means followed by the same letter are not significantly different (LSD; P=0.05).

† Trial 1, 7 DAT; Trial 2, 25 DAT; Trial 3, 2 DAT

‡ Trial 1, 99 DAT; Trial 2, 45 DAT; Trial 3, 12 DAT

Table 19. Affects of sediment type, application timing, and application method on efficacy of imidacloprid (0.5 lb a.i./ac) against borrowing shrimp (% reduction in burrows in treated compared to untreated plots). Each row represents a separate experiment.

Sediment Type / Timing	Burrow Density in Untreated Plots (#/m <sup>2</sup> )	Percentage burrow reduction		
		Spikewheel on ATV	Spikewheel on Boat	Broadcast
Sand / April	24	16		62
Sand / May	24	72		62
Sand / July	24	83		96
Sand / September	24	25		95
Silt / June	79		0	49
Sand / June	18		0	96
Eelgrass on sand / August	11	48	74	37



Other trials that featured application by spikewheel lacked a comparison with a broadcast application were conducted on beds with a thin eelgrass cover (Table 20). These trials demonstrated moderate to poor reduction in burrow density, with generally lower efficacies when applications were in August.

2) Large scale commercial trials, 2008

a) Methods

(1) Applications

Applications were made according to a Federal Use Permit and accompanying experimental label approved by the EPA. Both contained Directions for

Use and Restrictions that were similar to those in the 24C label for use of the standard material, Sevin™, on oyster beds (i.e., do not harvest clams or oysters within one year after treatment, proper and visible flagging of beds, a 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray, during aerial applications, all public access areas within one-quarter (¼) mile and all public boat launches within a one-and-a-half (1½) mile radius of any bed scheduled for treatment shall be posted). The experimental treatments were applied as similarly as possible to those made for the conventional carbaryl-based program and required the collaboration of the commercial applicator, Dan Foster, and the director of the carbaryl program, Dennis Tufts.

Table 20. Affects of application timing on efficacy of imidacloprid applied using spikewheels on ATV(0.5 lb a.i./ac) against burrowing shrimp (% reduction of burrows in treated compared to untreated plots). Each row represents a separate experiment.

Burrow Density in Untreated Plots (#/m <sup>2</sup> )	Percentage burrow reduction	
	July	August
12	83	
13	87	
29		17
11		72
28		0

Imidacloprid was applied aerially using helicopters to 7 commercial shellfish beds on July 2, 2008 in conjunction with applications of the Sevin, which was applied on July 2, 3, or 7 depending on bed location (Figure 5). Experimental beds were proposed by grower collaborators and selected based on degree of shrimp infestation, size, and proximity to untreated areas or beds treated with Sevin. A 20 ac bed located near the mouth of the North River (A90) had been fallow for at 12 years, had a moderate to heavy shrimp infestation and was isolated from other shellfish beds, so provided a good site to study both efficacy and non-target impact to salmonids. A 10 ac bed near the mouth of the Cedar River (A40) was also used as a site to assess both non-target impact and efficacy. A105 was located in between these sites and had the additional advantage of being accessible from shore. Two smaller beds were located in the Stoney Point growing area (B242 and B183). Two beds were also located in the Oysterville and Nahcotta growing areas (E148 and E163, respectively) where substrate is sandier than the primarily silty substrate of the northern and eastern areas of Willapa Bay. The original intent to match all beds with a nearby untreated area could not always be met. All beds except A105 were inspected prior to application for burrow density, dominant substrate type, amount and kind of eelgrass cover, and other attributes (Table 21).

Table 21. Attributes of commercial oyster beds treated with imidacloprid in Willapa Bay, 2008.

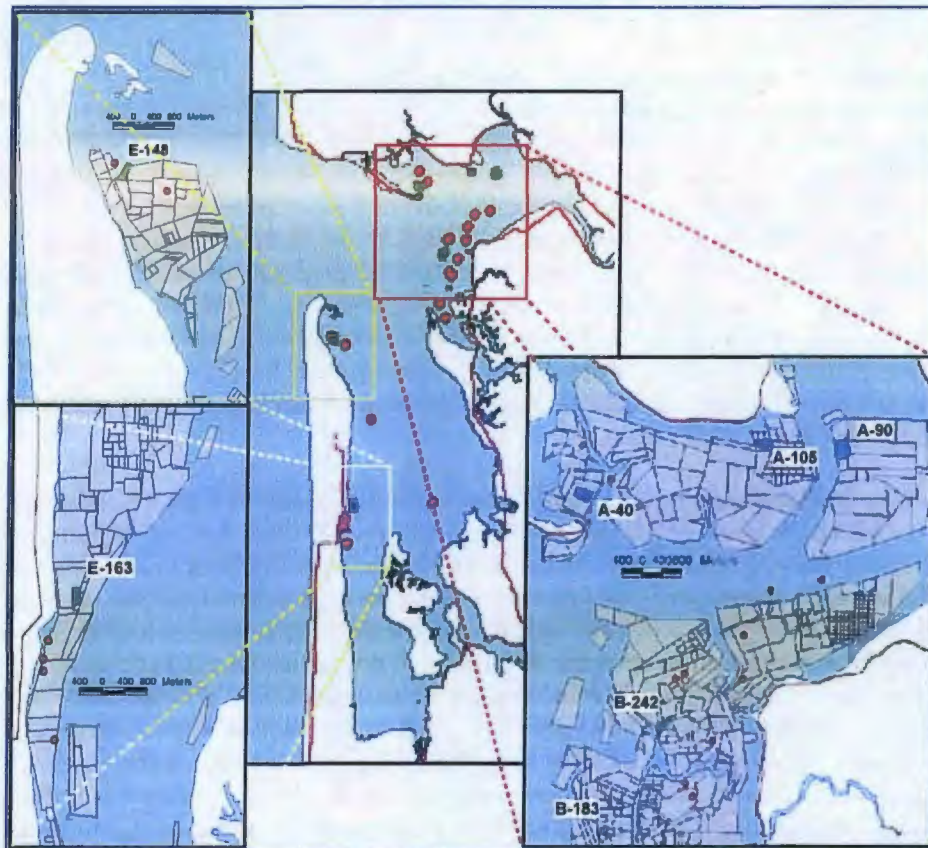
COMPANY	BED NAME	SIZE (ac)	STAGE <sup>a</sup>	LAST TRT <sup>b</sup>	PLANT DATE <sup>c</sup>	ELEV <sup>d</sup>	SP <sup>e</sup>	CUL T <sup>f</sup>	SUB <sup>g</sup>	EEL GRASS <sup>h</sup>	LAT <sup>i</sup>	LONG <sup>j</sup>
Nisbet Oyster	A40	10.0	Cedar R	2006	2008	0.3	G/M	S/H	S/I	heavy †	46.71417	-123.95542
Coast Seafood	A105	10.0	Cedar R	2002	2008	-0.5	M/G	S/H	M/I	heavy †	46.72493	-123.93408
Taylor Shellfish	A90	20.0	Cedar R	pre-95	none	-0.5	G/M	S/H	S/I	patchy †	46.43240	-123.53940
Nisbet Oyster	B242	6.0	Cedar R	2005	2007	1.0	G	S/H	S/I	none †	46.67035	-123.94487
Nisbet Oyster	B183	4.0	Cedar R	2005	2008	1.5	G/M	S/H	M/I	patchy †	46.65178	-123.95228
Northern	E148	10.0	Sheldon	½-'03, ½-none	2008	1.0	G/M	S	G/M/S	50% ‡	46.61520	-124.04040
Taylor Shellfish	E163	10.0	Sheldon	never	2008	0.5	G	S/H	S	patchy †	46.51505	-124.01963

<sup>a</sup> Helicopter staging area, <sup>b</sup> Year last treated, <sup>c</sup> Year and type of planting, <sup>d</sup> Bed elevation, <sup>e</sup> Species of shrimp (G-ghost, M-mud, G M-ghost dominant, M G-mud dominant), <sup>f</sup> Cultural Type (S-seed, H-harvest, LL-long line, <sup>g</sup> Substrate (M-Mud, S-Sand, I-Silt, G-gravel), <sup>h</sup> approximate density of either (†)native (*Zostera marina*) or ‡ Japanese (*Z. japonica*) density, <sup>i</sup> Latitude (decimal degrees), <sup>j</sup> Longitude (decimal degrees)



Imidacloprid was applied at a rate of 0.5 lb a.i. per ac to 5 of the 7 beds. Due to a mistake, beds in the Oysterville and Nahcotta growing areas were treated at 0.25 lb a.i. per ac. To test the affects of a second half-rate treatment, one half of Bed E163 was treated again 5 days later on July 7.

Two types of ground applications were also tested on the E163 bed: 1) subsurface injection using five Spikewheels™ pulled behind an Argo™ Track ATV and 2) application using 27' spray boom, also mounted on the Argo. Plot sizes were 2 and 5 ac, respectively. Application rate was 0.5 lb a.i. per ac on 1 August.



**Figure 5.** Name, location, size and shape of commercial oyster beds treated with imidacloprid (green) relative to locations of beds treated with carbaryl (red circles indicate points of entry).

### (2) Observations of burrowing shrimp

At all but one site, shrimp burrows were counted both before and at 4 weeks after treatment within a square meter grid placed along transects that criss-crossed the bed diagonally at distance intervals of 5, 10, or 15 paces depending on plot size, to give samples of 30 or more counts per bed. High flood tides sometimes constrained sample size. Counts were averaged within each half transect for statistical analysis.

### (3) Observations of impact to non-target macrofauna

Number of live, dead, or otherwise impaired but visible macrofauna were counted along transects at 5 shellfish beds following the applications. The area at each observation point was roughly 4 m<sup>2</sup> (2 m<sup>2</sup> to the front right and left plus 2 m<sup>2</sup> to the rear right and left). The entire bed could not be covered due to time limitations, but the transects usually crossed the beds diagonally so observations were made at both low and



high ends and at both sides. The number of paces between observation points, and consequent total number of observations, varied according to bed size and duration of the low tide. Three beds, two treated with imidacloprid and one treated with carbaryl, were examined within 1 hr after application. An untreated area near one of the imidacloprid-treated beds that was of similar bed elevation, substrate type, and vegetation cover was also examined as a check. Five beds (2 treated with imidacloprid, 2 treated with carbaryl, and the same untreated bed neighboring the imidacloprid-treated bed) were examined at 24 hrs after treatment.

#### (4) Water samples

Water was sampled for analysis of imidacloprid concentration directly on the bed of three beds and in the adjacent channels of two beds. On-bed samples were taken by grab near the center of the bed, initially when depth of the in-coming tide reached 6" and on subsequent high tides at mid-depth of the water column. In-channel grab samples were taken at both maximum low and high tides at mid-depth of the water column. All samples were held on ice and extracted for imidacloprid analysis within 7 days by Pacific Agricultural Laboratories, Portland, OR.

### b) Results

#### (1) Burrowing shrimp

Burrow density varied substantially at all aerially treated beds, both before and after treatment with imidacloprid (Figure 6). In general, burrow density was significantly lower in beds after treatment with imidacloprid, but levels were not low enough to allow oysters to survive. At the A90 site, burrow density declined significantly from 13.9 at 14 days before treatment to 8.1 at 29 days after treatment (DAT) but was high again 30 days later at 59 DAT. Burrow density also declined in the first 29 DAT, although not significantly, in the nearby untreated area. Due to its drainage patterns and proximity to the North River and a major channel, A90 had a much less regular surface than most other shellfish beds. Burrows on the myriad of small hummocks had been exposed for longer and were much more visible than burrows under

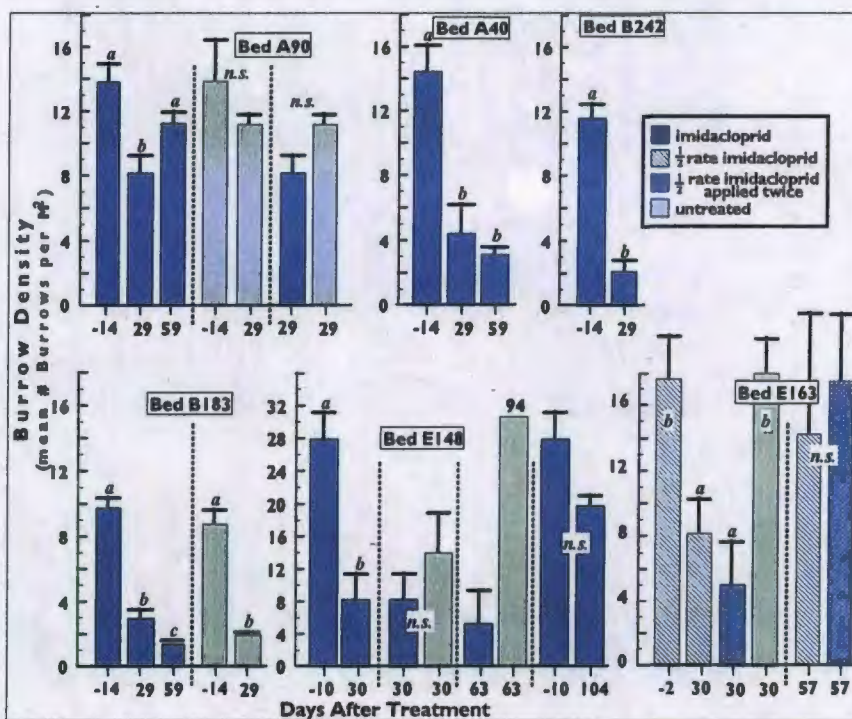


Figure 6. Effects of imidacloprid on burrowing shrimp density at 2, 10, or 14 days before and at 4, 8, or 10 weeks after treatment. Letters "a" and "b" indicate significantly different densities (n.s., not significant).



water. At 58 DAT, mean burrow density on exposed ground was 12.1 compared to 8.9 on ground under  $\frac{1}{2}$  or more inches of water. At A40, number of burrows per  $m^2$  apparently declined to an acceptable level (4.4 burrows/ $m^2$  at 29 DAT and 3.1 burrows/ $m^2$  at 58 DAT), but heavy covers of native eelgrass and algae complicated assessments and could have caused some burrows to be missed. The lack of an adequate untreated control site near A40 also confounded interpretation of results. A similar scenario occurred at B242: burrow density apparently declined significantly and to a potentially acceptable level after treatment with imidacloprid, but heavy vegetation and the lack of a nearby untreated area for comparison confounded the experiment. At B183, burrow density declined in the bed treated with imidacloprid, but also declined in a nearby untreated area in the first 29 DAT. However, the check at B183 was close enough to the treated area that it could have been contaminated by off-site drift. Bed E148, treated with the half rate of imidacloprid, initially showed a similar scenario as that at the A90 site: burrow density was significantly lower at 30 DAT compared to 10 days before treatment, but was still not at an acceptable level for planting. Burrow density was measured as lower at 63 DAT, but not all sections of the bed were examined. A more thorough examination of the bed at 104 DAT gave a higher burrow density.

Shrimp burrow density was also quite variable within beds, especially post-treatment. Some portions of the bed showed moderate burrow density, but other sections were nearly barren. At the first post treatment assessment, comparisons of burrow densities along the transects at some beds showed relatively highly variable post-treatment distributions of shrimp burrows, especially at E163 (Figure 7). At Bed A148, four strips of relatively low burrow density (9.2, 8.3, 1.8, 1.7 per  $m^2$  at a third post-treatment assessment (58 DAT)) were interspersed among stretches of higher burrow density (not counted). Burrow densities at a nearby untreated site were significantly higher (94.4 per  $m^2$ ).

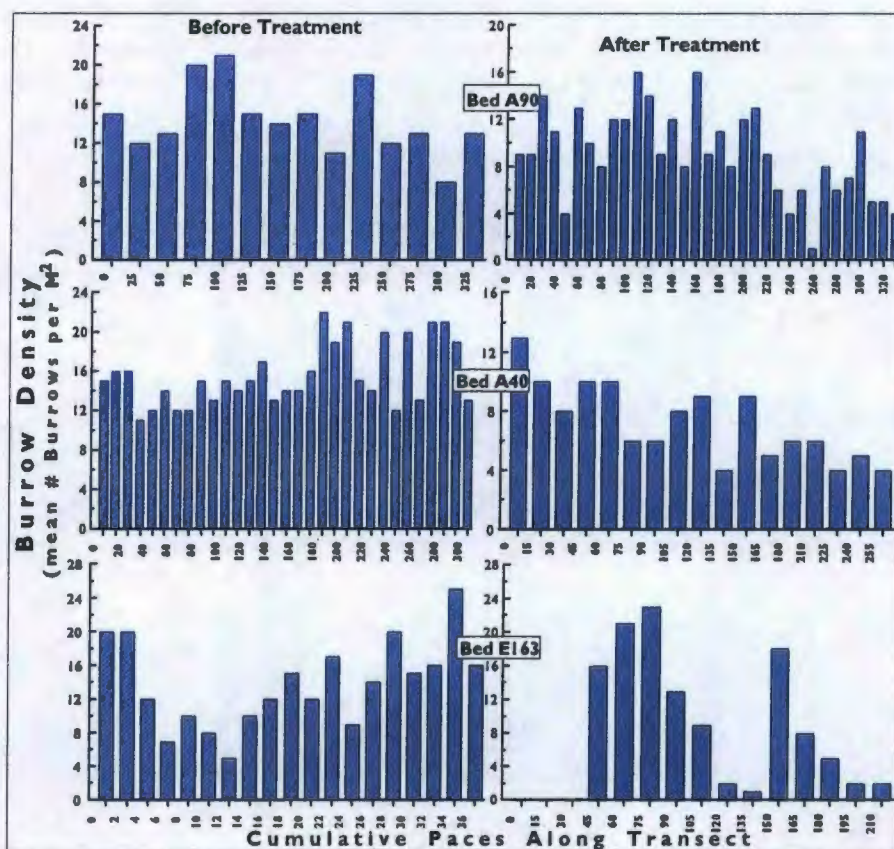


Figure 7 Variation in burrow density along sampling transects at the beds treated with imidacloprid.



Additional observations at E163 showed the patchy distribution of burrow counts to be associated with vegetation, substrate elevation, and related patterns of tidal drainage (Figure 8). At Bed A148, four strips of relatively low burrow density (9.2, 8.3, 1.8, 1.7 per m<sup>2</sup> at a third post-treatment assessment (63 DAT)) were interspersed among stretches of higher burrow density (not counted). The width of these strips (~18 ft) is similar to the width of a spray strip. Burrow densities at a nearby untreated site were significantly higher (94.4 per m<sup>2</sup>).

The ground applications at E163 showed significant reductions in burrow densities in plots treated using either Spikewheels or spray boom compared to both pretreatment levels and densities in an adjacent untreated plot (Figure 9).

(2) Impact to non-target macrofauna, primarily crab

No visibly affected fish were observed. Although a few dead nereid polychaetes were observed at the A90 and the E163 beds, crabs (Dungeness, rock, and hermit) were observed as the most primary animal impacted by imidacloprid (Table 22).

Affected crabs were not dead, but in a state of chronic tetanus shock. They were either entirely exposed or only partially buried and moved very sluggishly when disturbed. Legs and mouthparts were extended and trembled constantly. In comparison, more crab were affected on beds

treated with carbaryl and all were dead. Almost all crab were observed in lower areas of the bed or off-bed.

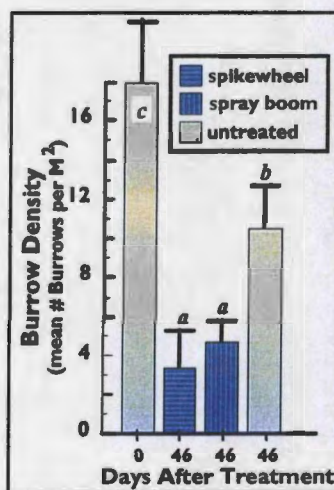


Figure 9 Burrow density in large plots treated with imidacloprid using spikewheels or spray boom.

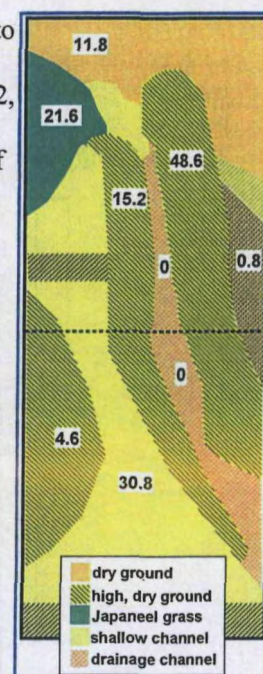


Figure 8 Distribution of burrow counts among bed attributes at E163 and 63 DAT.

Table 22. Impact of imidacloprid (imid), carbaryl, or no treatment (untreated) on crab, as observed visually at 1 or 24 hours after treatment (HAT).

Bed	Treatment	Treatment Date	HAT	Transects	Paces Between Observations	Observations	Number Crab		
							Normal	Tetanus	Dead
A90	imid	July 2	1	3	1	500	0	0	0
A91	untreated		1	3	1	683	0	0	0
A40	imid	July 2	1	4	5	146	0	0	0
E147	carbaryl	July 7	1	5	1	500	0	0	3
A90	imid	July 2	24	6	5	204	0	15	0
A91	untreated		24	2	5	46	0	0	0
A40	imid	July 2	24	4	5	79	0	6*	0
B183	imid	July 2	24	2	5	65	2	3**	0
E163	imid	July 2	24	7	1	700	0	1	0
A100	carbaryl	July 7	24	4	10	69	3	0	100***
A79	carbaryl	July 7	24	3	20	60	0	0	25****

\* also 10 – 15 lethargic and attenuating crab submerged in drainage channel off lower end of bed.

\*\* also 4 – 8 lethargic and attenuating crab submerged in drainage channel off bed.

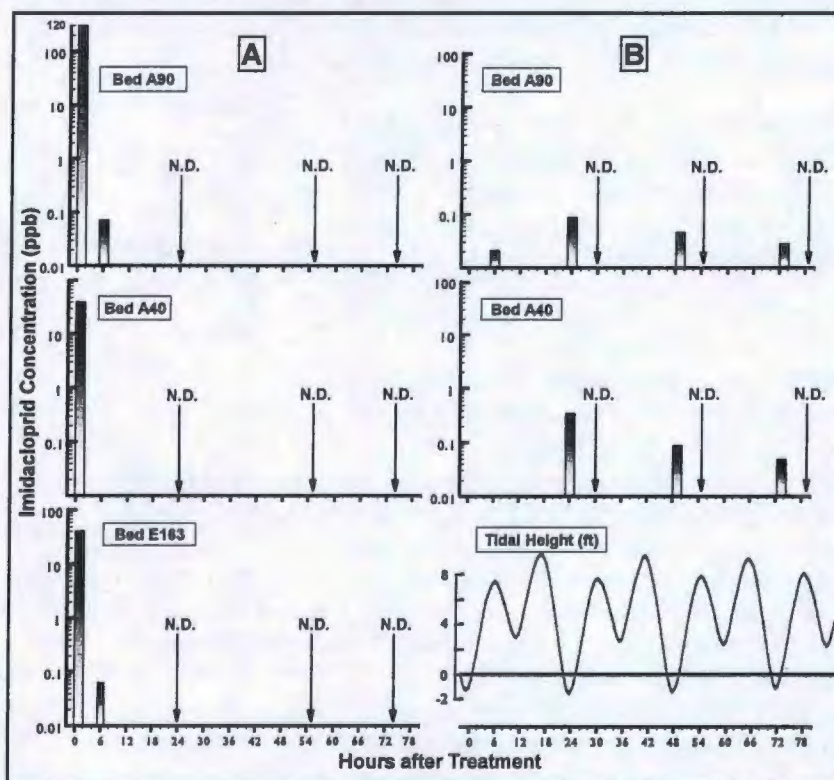
\*\*\* also ~ 100 dead crab in 3×5 m section of drainage channel off lower end of bed.

\*\*\*\* rapidly rising tide prevented off-bed observations.



## (3) Water Samples

Concentrations of imidacloprid sampled over the beds dropped precipitously between 1 and 6 hours after treatment (HAT) and were not detected afterward (Figure 10). Concentrations in the channels adjacent to the beds were recovered from both sample sites at 6 and 24 HAT and at 49 and 74 HAT at one of the sites. These timings were synchronized to the high tides.



**Figure 10.** Concentrations of imidacloprid in water sampled (A immediately over the bed, B) in adjacent channels after applications at ~6 a.m. July 2 and C) tidal fluctuations during the same time at Toke Point near Beds A90 and A40. N.D., not detected (Method Reporting Limit = 0.02 ppb).

## c) Discussion

The general failure of the aerial applications of imidacloprid to suppress burrowing shrimp densities to commercially acceptable levels was due to several factors. The water samples indicated that at least some imidacloprid was transported off-bed during high tide, which likely contributed to generally poor on-bed efficacy against burrowing shrimp relative to carbaryl. Imidacloprid has a lower coefficient of adsorption than carbaryl, so does not bind as tightly to sediments, especially silt, a major component of Willapa Bay tidelands. In addition, most of the beds where efficacy was poor were blanketed with thick vegetation which likely inhibited penetration of imidacloprid. Percent cover of native eelgrass (*Zostera marina*) averaged 67% on Bed A40 and 47% on Bed B183 during pre-treatment assessment while average percent cover of Japanese eelgrass (*Z. japonica*) was 37% on E163. Cover of eelgrass and sea lettuce (*Ulva sp.*) increased during late summer and frequently exceeded 100% in many of the m<sup>2</sup> grids, which greatly confounded measurement of shrimp burrows. At A90, the currents from the North River may have contributed to the already strong tidal currents to wash imidacloprid from the bed before kill. Rising tides approach B183 from both east and west so imidacloprid may not have been washed away as quickly there, resulting in relatively better efficacy. B183 had also been recently dredged so may have retained imidacloprid longer. Impact to non-target macro-fauna was mostly limited to crab and apparently to a smaller portion of the on-bed population compared to carbaryl.



## 3) Small plot trials, 2009

Early season trials have focused on the affects of higher rates and different formulations than the 2F formulation (Nuprid™, NuFarm Inc) at 0.5 lb a.i./ac. Results so far indicate that 5% and 1% granular formulations of imidacloprid (Mallet 0.5G™ and Mantra 1G™, respectively, both NuFarm Inc.) to be highly effective, both alone and when combined with reduced rates of carbaryl (Sevin 80S™, Bayer Corp.) (Tables 23, 24).

Table 23. Affects of imidacloprid formulated as a 5% or 1% granular (Mallet 0.5G, Mantra 1G, respectively) or 2 lb/gal flowable (Nuprid 2F) applied alone or in combination with an 80% wettable powder formulation of carbaryl (Sevin 80WP) on burrowing shrimp (# burrow / m<sup>2</sup>).

Treatment	Rate (lb a.i./ac)	Burrow Density*	
		Pre-treatment †	Post-treatment ‡
Mallet 0.5G	2.0	44.4 <i>n.s.</i>	0.2 <i>a</i>
Mallet 0.5G	1.0	53.2	1.2 <i>a</i>
Mallet 0.5G	0.5	49.6	0.3 <i>a</i>
Nuprid 2F	0.5	56.0	1.2 <i>a</i>
Nuprid 2F	1.0	57.2	0.5 <i>a</i>
Nuprid 2F	2.0	50.8	0 <i>a</i>
Nuprid 2F+Sevin 80WP	0.5 / 2.0	51.2	2.3 <i>a</i>
Nuprid 2F+Sevin 80WP	0.5 / 4.0	50.8	0.3 <i>a</i>
Mantra 1G	1.0	360	0.2 <i>a</i>
Untreated	0	49.2	38.7 <i>b</i>

\* means followed by the same letter are not significantly different (LSD; P=0.05).  
† 4 days before treatment, 4/23/09  
‡ 8 days post treatment, 5/6/09

Table 24. Affects of formulation and rate of imidacloprid on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 3 trials and at 10 – 12 days after treatment at Ellen Sands (Trial 1), Sherwood (Trial 2), and WDFW (Trials 3,4), Spring 2009.

Trial	Treatment	Rate (lb a.i./ac)	Burrow Density*	Comments
1	Nuprid 2F	2.0	3.2 ± 0.8 <i>a</i>	sandy, silt substrate
	Mallet 0.5G	0.50	20.4 ± 3.2 <i>a</i>	
	Untreated	0	96.4 ± 2.8 <i>b</i>	
2	Nuprid 2F	2.0	7.6 ± 0.8 <i>a</i>	sandy, silt substrate light eelgrass know dry time, fast flood tide
	Mallet 0.5G	0.50	4.0 ± 1.2 <i>a</i>	
	Untreated	0	31.2 ± 1.2 <i>b</i>	
3	Nuprid 2F	2.0	26.4 ± 2.4 <i>a</i>	sandy, silt substrate tidal flow 2F in water (2 – 5")
	Mallet 5G	0.50	27.6 ± 2.0 <i>a</i>	
	Untreated	0	78.8 ± 2.4 <i>b</i>	
4	Nuprid 2F	2.0	5.2 ± 1.2 <i>a,b</i>	sandy, silt substrate thick eelgrass cover wet plots / standing water
	Mallet 0.5G	0.50	14.6 ± 2.0 <i>a</i>	
	Untreated	0	30.8 ± 1.6 <i>b</i>	
5	Nuprid 2F	2.0	14.2 ± 1.2 <i>a</i>	sandy, silt substrate thick eelgrass cover wet plots / standing water
	Mallet 0.5G	0.50	27.6 ± 2.0 <i>a</i>	
	Untreated	0	30.8 ± 1.6 <i>b</i>	

\* means followed by the same letter are not significantly different (LSD; P=0.05).



**F) Petition for Temporary Tolerance**

An exemption from setting a temporary tolerance is requested in this EUP application based primarily on two lines of argument.

The first argument rests on EPA's own discussions regarding tests for determination of "Accumulation in Laboratory Fish (165-4)". The agency explicitly stated the data requirement was waived for the following reason: "Octanol/water partitioning ( $K_{ow}$ ) data provided by the registrant implies a low potential to bioaccumulate ( $K_{ow}$  for imidacloprid = 3.7 @21 °C)" (Parker 2006). These statements imply that the agency review determined that depuration of residues would be very fast and bioconcentration would thus be minimal, especially as concentration following exposure would be widely fluctuating. The rodent metabolism study showed over 90% dissipation of radiolabelled compounds within 24 h suggesting that biological metabolism across species ought to be equally as fast. There is no reason to expect that oysters would not process imidacloprid efficiently as observed in rodents, nor is there any reason to suspect that the bioconcentration or bioaccumulation factor would be significantly different from that predicted for fish.

The second line of argument in favor of a temporary tolerance exemption for this EUP comes from a modeling exercise. A fugacity based model titled FISH Model (version 2, November 2004) is available in the public domain from the Canadian Environmental Modeling Center at Trent University, Peterborough, Ontario, Canada (<http://www.trentu.ca/academic/aminss/envmodel/models/Fish2.html>). The model uses a combination of chemical physicochemical properties and several fish pharmacokinetic parameters to predict whole and lipid fish tissue residue concentrations given a starting point for residues in the water column. Bioaccumulation of chemicals by fish includes both absorption through the gills and food ingestion. The default fish parameters represents fitted parameters from studies with guppies, goldfish, and rainbow trout (Clark et al. 1990). The following analysis makes the assumption that oyster toxicokinetics is similar to that of the default fish model represented in FISH.

The FISH Model was run under two scenarios based on estimated water concentrations and default toxicokinetic assumptions.

First, two imidacloprid water concentrations were used as model input, along with the default fish toxicokinetic parameters: 1) 36 µg/L, the EDWC (estimated drinking water concentration) from EPA's drinking water assessment for imidacloprid yield by the FQPA Index Reservoir Screening Tool (FIRST) (Parker 2006), and 2) 33 µg/L, based on the 0.5 lb a.i./ac, application rate proposed in this EUP application. The application rate was adjusted for depths of water ranging from 1-10 foot. This adjustment is based on Willapa Bay tidal cycles. NOAA data shows a water level change approximating 1.6 ft/hr from low to high tide and back to low tide (<http://tidesonline.noaa.gov/geographic.html>). The highest concentration therefore was estimated to be ~184 µg/L when the water depth was at one foot. The average concentration based on the application rate relative to the algebraic average of all depths during one tidal cycle was 33 µg/L. This concentration was slightly lower than the EWDC from FIRST modeling and thus would hardly change the ultimate exposure perspective.

Second, two default toxicokinetic assumptions in the FISH Model were increased by a factor of 10-fold to increase uptake of imidacloprid by fish and therefore conservatively bias the model output for higher tissue residues. Specifically, the food intake rate was increased from 2% of body weight to 20% of body weight, and the gill resistance factor for the organic phase was reduced from 300 h to 30 h. The other default parameters were not changed. The input water concentrations were the same as in the first scenario (i.e., 36 µg/L and 184 µg/L). Our application of the the FISH Model used imidacloprid concentrations in fish that ranged on a whole body basis from 0.814 µg/kg to 21.1 µg/kg (the assumed body tissue density was 1 kg/L). To estimate exposure, a 0 – 5 year old child was assumed to eat 162 g/day of fish (EPA



Child-Specific Exposure Factors Handbook 2002). This rate was based on the highest mass of fish consumed as recorded in the Columbia River Intertribal Fish Commission study. Exposure estimates, based on a 10-kg child, ranged from 0.0000132 mg/kg/day -- 0.0003418 mg/kg/day.

To characterize the incremental increase in risk that the estimated exposures represented, dietary (food and drinking water) and residential exposure were aggregated. EPA's estimate of aggregate food and drinking water acute exposure was nearly three-fold higher than the chronic exposure value, so it was used in subsequent analyses. For residential exposure, a child with short-term (1-30 day) exposure to a pet treated with imidacloprid was estimated to be higher than other exposure scenarios. EPA did not conduct an intermediate or long-term residential exposure owing to lack of significant hazard in rodent chronic toxicity studies.

The total aggregate exposure was estimated to be 0.15643 mg/kg/day (0.09761 mg/kg/d for dietary/drinking water exposure and 0.05882 mg/kg/day for residential exposure, based on back calculation from an estimated MOE of 170 and a NOEL of 10 mg/kg/day).

The percentage contribution of putative fish tissue exposure was calculated to range from 0.0084% at the low end to 0.2185% at the high end of water residues. Thus, the contribution of fish tissue residues of imidacloprid (and presumably oyster tissues) would not change the overall aggregate risk characterization of imidacloprid.

**In consideration of the EPA's discussions regarding accumulation on imidacloprid accumulation in fish, the results of the FISH model, other observations regarding potential exposure risks (Section D. Residue Data, above), and the isolated location of treated beds (Section G. Proposed Experimental Program, below), we request an exemption from tolerance.**

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**G) Proposed Experimental Program****1) Qualifications and Identifications of Participants****a) Researchers***Dr. Kim Patten*

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**Selected Recent Publications:**

Patten, K and C. O'Casey 2007. Use of Willapa Bay, Washington, by shorebirds and waterfowl after Spartina control efforts. J. Field Ornithol. 78(4):395-400

Patten, K. 2006. Review of Clearcast (Imazamox) Aquatic EUP and research results for the western U.S. Proceedings of Aquatic Plant Management Society. August, 2006.

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mosquitoes on aquatic invertebrate communities, and the effects of pesticides in surface waters on the survival and reproduction of salmonids. He teaches a class in fish and wildlife toxicology. Dr. Grue is an active member the Society of Environmental Toxicology and Chemistry and the Wildlife Society and frequently serves on advisory panels dealing with pesticides and other environmental contaminants. He has recently served on FIFRA Science Advisory Panels, the Five-year Review Committee for the USGS's Contaminant Biology Program, and the Editorial Board of the Bulletin of Environmental Contamination and Toxicology, and was recently appointed to the External Advisory Group for the Washington Department of Ecology dealing with the agency's permit for aquatic weed control and eradication.

Selected Recent Publications:

- Grue, C.E., S.C. Gardner and P.L. Gibert. 2002. On the significance of pollutant-induced alterations in the behavior of fish and wildlife. Chapter 1 (pages 1-90) in G. Dell'Omo (ed.) Behavioural Ecotoxicology, John Wiley & Sons, Ltd., West Sussex, UK.
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Food and Environmental Quality Lab  
Richland, WA

Degrees:

Undergraduate: 1983	Masters: 1987	Ph. D.: 1999
Humboldt State University	University of Nevada	University of Nevada
Arcata, CA	Reno, NV	Reno, NV

Areas of active research: 1) developing analytical methods for assessing specific biomarkers useful for monitoring pesticide exposures to sensitive subpopulations in agricultural communities, 2) the development of field air -sampling methods and volatilization chamber

system design for assessing fumigants, pesticides, and semiochemicals useful in codling moth mating disruption, 3) characterizing/isolating bioactive plant volatile emissions from insect herbivory that may prove useful in enhancing conservation biological control in cropping systems, and 4) chemically assessing sublethal concentrations of pesticides in surface waters that can have neurobehavioral effects on salmonids. A principle responsibility is to administer over a state-mandated food and environmental regulatory science facility that conducts studies under federal 40CFR Part 160 Good Laboratory Practices (GLP). This program houses an independent quality assurance unit and GLP Laboratory Coordinator to assure federal compliance.

#### Selected Recent Publications:

- Hebert VR and Miller GC. Understanding the tropospheric fate of agricultural pesticides, in *Reviews of Environmental Contamination and Toxicology*, ed. G. Ware, Vol. 181 pp 1-36 (2004).
- Woodrow J, Hebert VR, LeNoir J. "Monitoring Of Agrochemical Residues In Air." in "Handbook of Residue Analytical Methods for Agrochemical Residues" (P. Lee ed., two volume series) John Wiley & Sons. pp. 908-935 (2003).
- Merriman J, Hebert VR Methyl Isothiocyanate Residential Community Air Assessment; South Franklin County, Washington. *Bull of Environ Contam and Toxicol*. In press (Jan 2007)
- Hebert, VR. Understanding the tropospheric transport and fate of semivolatile pest management chemicals. In: *Environmental Fate and Safety Management of Agrochemicals ACS Symposium Book Series 899*, ed. JM Clark, pp 70-82 (2005).
- Hebert, VR, Hoonhout C, Miller GC. Reactivity of certain gas-phase organophosphorus insecticides toward hydroxyl radicals at elevated air temperatures. *J. Agric. Food. Chem.*, Vol. 48: (2000): 1922-1928.
- Hebert, VR, E Tomaszewska, J. F. Brunner, V. P. Jones, and M. Doerr. Evaluating the pheromone release rate characteristic of commercial mating disruption devices. In *Crop Protection Products for Organic Agriculture. Environmental, Health, and Efficacy Assessment*. Felsot, A.S., K. D. Racke (ed.); Am. Chem. So, Symposium Series 947, Am. Chem. Soc., Washington, DC. pp. 144-157 (2006).
- Weppner, S, Elgethun K, Lu C, Hebert VR\*, Yost M, Fenske R. The Washington aerial spray drift study: Children's exposure to methamidophos in an agricultural community following fixed-wing aircraft application *J. Expos. Anal. Environ. Epidem* 16: 387-396 (2006).

#### *Dr. Alan Felsot*

Professor and Extension Specialist  
Entomology and Environmental Toxicology  
Washington State University-Tri Cities  
Food and Environmental Quality Lab  
Richland, WA

#### Degrees:

Undergraduate: 1972	Masters: 1974	Ph. D.: 1978
Tulane University	University of Florida	Iowa State University
New Orleans, LA	Gainesville, FL	Ames, IA

Research and Extension Interests: Hazard assessments of transgenic crops, pesticide drift and buffer zone design, reduction of insecticide application rates using new sprayer technologies, enhanced biodegradation of pesticides, remediation of pesticide waste in soil, best management practices for controlling agrochemical movement to surface and ground water, analytical chemistry of pesticide residues in soil, water, and food, pesticide toxicology, regulations, and risk communication. He teaches a graduate course entitled "Applied Environmental Toxicology." He also team teaches the course, "Pesticides: Toxicology and Modes of Action."

#### Recent Publications:

- Felsot, A. S. 2004. Establishing buffers: Protocols and toxicological benchmarks, *Proc. International Conference on Pesticide Application for Drift Management*. Oct 27-29, Waikoloa, HI. pp. 199-203.
- Felsot, A. S. 2004. Impact of U.S. court cases on application technology, *Proc. International Conference on Pesticide Application for Drift Management*. Oct 27-29, 2004, Waikoloa, HI. pp. 53-58.
- Felsot, A. S. 2004. Is the content of disease-reducing phytochemicals influenced by certified organic



crop production practices? Paper no. 21, 228th National Mtg. American Chemical Society (PICOGRAM Issue no. 67, p. 55), Aug 22-26, 2004. Philadelphia, PA.

Ramaprasad, J., M.-Y. Tsai, K. Elgethun, V. R. Hebert, A. Felsot, M. G. Yost, R. A. Fenske. 2004. The Washington aerial spray drift study: assessment of off-target organophosphorus insecticide atmospheric movement by plant surface volatilization. *Atmospheric Environment* 38:5703-5713.

Felsot, A. S., 2004. No-spray buffer zones for the ag/urban interface: derivation using drift modeling and toxicologically relevant benchmarks (26 MB \*.pdf). Paper no. 85, 227th National Mtg. American Chemical Society (PICOGRAM Issue no. 66, p. 68), Mar 28-Apr 1, 2004. Anaheim Calif.

#### b) Consultants

*Dr. Alan Schreiber*

President, Agriculture Development Group, Inc., Pasco Washington  
 Administrator - Washington State Commission on Pesticide Registration  
 Executive Director - Washington Asparagus Commission

#### Degrees:

Undergraduate: 1984	Masters: 1987	Ph. D.: 1991
Northeast Missouri St. Univ.	University of Missouri	University of Missouri
Kirksville, MO	Columbia, MO	Columbia, MO

Research and Extension Interests: For the Ag Development Group, Dr. Schreiber consults on environmental, pesticide, pest management and Food Quality Protection Act issues for grower groups, governmental organizations and agribusiness's and conducts research on more than 30 crops on a 100 acre research farm. For the WSCPR, a state governmental entity dedicated to support of activities related to pesticide registration and pest management, Dr. Schreiber manages a \$0.9 million budget and interacts with all commodity and pest management groups, pest management researcher and extension specialist in Washington. Prior to these positions, Dr. Schreiber was Assistant Professor for the Department of Entomology, Washington State University, and before that, Entomologist for the USEPA/Office of Pesticide Programs/Biological and Economic Analysis Division

#### Honors and Awards:

Outstanding Service Award to U.S. Potato Industry, National Potato Council, 2002  
 Entomological Society of America, Excellence in Extension nominee, 1997  
 WSU Outstanding Extension Scientist, Department of Entomology nominee,  
 1997 Oregon/Washington Asparagus Growers Assn. "Friend of the Industry Award,"  
 1996 Columbia Basin Vegetable Seed Association Outstanding Service Award, 1995

*Dr. Steven Booth*

PSI / WGHOGA  
 120 State St. NE #142  
 Olympia, WA 98501

#### Degrees:

Undergraduate: 1975	Masters: 1982	Ph. D.: 1992
University of Iowa	Western Washington Univeristy	Oregon State University
Iowa City, IA	Bellingham, WA	Corvallis, OR

Research and Extension Interests: As the IPM Coordinator for the Willapa Bay / Grays Harbor Oyster Growers Association, Dr. Booth assists in the development and implementation of a variety of chemical, biological, and mechanical tactics for the control of burrowing shrimp. He has writes grant proposals to fund the IPM program and reports that describe its progress. Prior to his current position, Dr. Booth has developed IPM tactics featuring biorational pesticides, insect parasitic nematodes and fungi, and beneficial insects.

#### Recent Publications:

Booth, S.R., Drummond, F. and E. Groden. 2007 Special considerations for application and evaluation

of entomopathogens in specific systems: Small fruits. *in* Field Manual of Techniques for the Use and Evaluation of Entomopathogens, 2<sup>nd</sup> Edition. [L. Lacey and H. Kaya, eds., Ch. VII.12. Kluwer Press. pp 583 – 598.

Dumbauld, B.R., Booth, S.R., Cheney, D., Suhrbier, A., and H. Beltran. 2006. An integrated pest management program for burrowing shrimp control in oyster aquaculture. *Aquaculture*. 261: 976-992.

Booth, S.R., Tanigoshi, L.K., and Shanks, C., Jr. 2002. Evaluation of entomopathogenic nematodes to manage root weevil larvae in Washington state cranberry, strawberry, and red raspberry. *Env. Entomol.* 31:895-902.

Booth, S.R., Tanigoshi, L.K., and I. Dewes. 2000. Potential of a dried mycelium formulation of an indigenous strain of *Metarhizium anisopliae* against subterranean pests of cranberry. *Biocontrol Science and Technology* 10:659-668.

Booth, S.R. and C.H. Shanks. 1998. Potential of a dried rice/mycelium formulation of entomopathogenic fungi to suppress subterranean pests in small fruits. *Biocontrol Science and Technology*. 8:197-206.

**c) Grower Cooperators – members of WGHOGA who own acreage allotments**

<i>Kristi Ballo</i> Brady's Oysters 3714 Oyster Pl. E. Aberdeen, WA 98520	<i>Nick Jambor</i> Ekone Oyster Co. 29 Holtz Road South Bend, WA 98586	<i>Jerry Swan</i> Grass Creek Oyster Co 1975 Lakemoore Pl SW Olympia, WA 98512
<i>Leonard Bennett</i> R&B Oyster Co P O Box 309 Bay Center, WA 98586	<i>James Kemmer</i> Long Island Oyster PO Box 1054 Long Beach, WA 98631	<i>Bill Taylor / Eric Hall</i> Taylor Shellfish Co., Inc. SE 130 Lynch Road Shelton, WA 98584
<i>Dan Driscoll</i> Oysterville Seafarms P O Box 6 Oysterville, WA 98641	<i>Tim Morris</i> Coast Seafoods Box 166 South Bend, WA 98586	<i>Dennis Tufts</i> Wilson Oyster Co. PO Box 236 Ocean Park, WA 98640
<i>Don Gillies</i> Stony Point Oyster Co. L.L.C. 6931 US Hwy 101 South Bend, WA 98586	<i>Dave Nisbet</i> Nisbet Oyster Co. PO Box 338 Bay Center, WA 98527	<i>Fritz Wiegardt</i> Wiegardt & Sons P O Box 309 Ocean Park, WA 98640
<i>John Heckes</i> Heckes Clam Co P O Box 1657 Ocean Park, WA 98640	<i>Phil Olsen</i> Olsen & Son Oyster Co. PO Box 212 South Bend, WA 98586	<i>Dr. Richard Wilson</i> Bay Center Mariculture P O Box 356 Bay Center, WA 98586
<i>David Hollingsworth</i> Markham Oyster Inc. 20 Old Westport Road. Aberdeen, WA 98520	<i>Brian Sheldon</i> Northern Oyster Company PO Box 1039 Ocean Park, WA 98640	

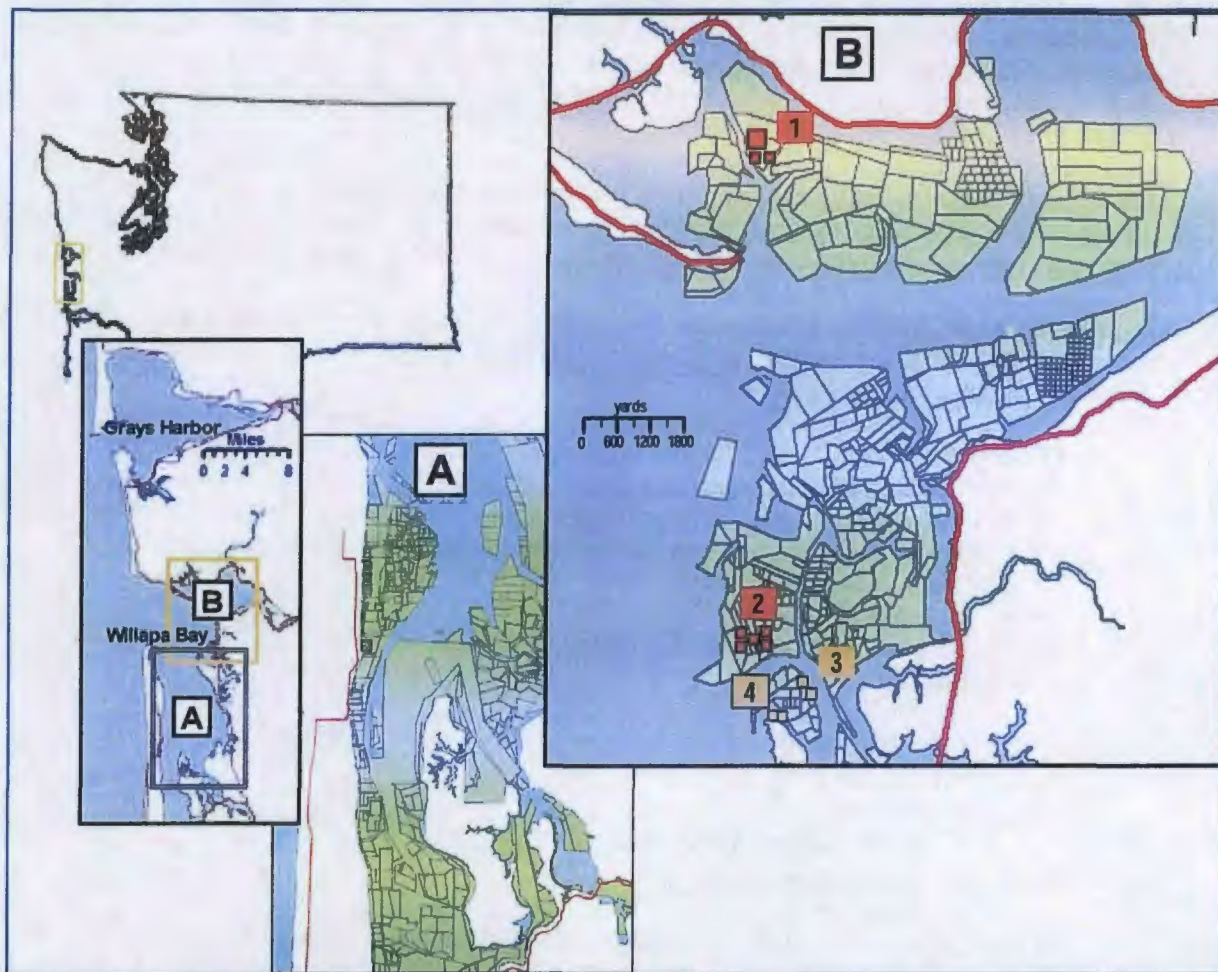
**2) Locations, acreage to be treated**

All areas to be treated lie within the 4,250 intertidal acreage of Willapa Bay and Grays Harbor (4250 ac (Feldman et al. 2000) and 7,500 ac (<http://graysharbor.fws.gov>), respectively). Most of the 35,000 commercial acreage (BSCC 1992) lie several hundred meters from land and human habitation. A range of 70 to 120 intertidal ac will be treated with imidacloprid. Treatments will feature different three treatment rates of liquid soluble concentrate imidacloprid (Nuprid 2F; NuFarm America, Inc.) (2.0, 1.0, and 0.5 lb a.i./ac) and two treatment rates of 0.5% granular imidacloprid (Mallet 0.5 G; NuFarm, Inc.) (1.0 lb a.i./ac and 0.5 lb a.i./ac). The maximum acreage represents four 5 ac plots of each rate / formulation combination plus one 10 ac plot of Nuprid 2F applied at 1.0 lb a.i./ac plus one 10 ac plot of Mallet 0.5G applied at 0.5



lb a.i./ac (Table 25). The minimum acreage represents one 5 ac plot plus two 2.5 ac plots for each formulation / rate combination plus one 10 ac plot of Nuprid 2F applied at 1.0 lb a.i./ac plus one 10 ac plot of Mallet 0.5G applied at 0.5 lb a.i./ac.

Due to annual variation in densities of burrowing shrimp, we cannot at this time (late summer 2009) predict where all experimental plots will be sited. However, two large areas of high shrimp density will likely be available, one near the Cedar River and the other north of the Bay Center Peninsula and the Palix River Channel (Figure 11). Beds will be selected based on density of burrowing shrimp, substrate type, grower cooperation, ease of access, size, proximity to beds targeted for carbaryl application, proximity to untreated areas, and proximity to known salmonid populations.



**Figure 11** Location of known potential sites for experimental application of imidacloprid in the Nahcotta (A) and Cedar River / Stony Pt / Palix River are in Willapa Bay in 2010.

#### Details of the Proposed Program

It is highly unlikely that we will be able to compare all formulation / rate treatments at each study site (e.g., a factorial experimental design) due to area limitations and a desire to minimize potential impact. Instead, selected treatment combinations will be more likely be compared in pairs or triplicates. Nuprid 2 F will be applied aerially using helicopters, as in the conventional carbaryl-based shrimp management program, or using a spray boom apparatus mounted on an ATV. Mallet 0.5G will likely be applied at 0.5 using conventional ground-based granular dispensers (e.g., belly grinders), but we may wish to apply at least one bed aerially using helicopters.



Application date will depend on the treatment schedule for the conventional carbaryl-based program. Imidacloprid will be applied on a separate day, preferably 2 or 3 days prior to the carbaryl treatments.

Burrowing shrimp densities will be counted on beds to be treated within 2 weeks prior to and at 4 -- 8 weeks after treatment by counting burrows inside a 1m<sup>2</sup> grid along transects that diagonally cross the bed or otherwise adequately represent the bed. Percentage cover of eelgrass, algae, shell, and standing water will also be recorded.

Trials and assessments of efficacy will be directed primarily by Dr. Kim Patten, Long Beach Research Unit, Washington State University and Dr. Steven Booth, Pacific Shellfish Institute. For both small plot and commercial scale trials, efficacy will be judged primarily by comparing shrimp burrow counts taken before treatment and at several post treatment intervals (~4 -- 8 weeks and, pending results, 11 months after treatment). On commercial beds, the length of the interval before sampling will also depend on when seed is planted. Walking on newly planted seed will substantially damage the crop. Efficacy on each bed will also be eventually and ultimately be judged by yield.

Ancillary studies related to non-target impacts depend on results from this year's study on a single 10 ac plot and cannot be detailed at this time. Studies to address impact to salmonids, sturgeon, crab, and the benthic in-fauna will be coordinated with state agencies and detailed in a later submission.

Objectives and methods of the current studies are described in the current experimental study plan currently under execution via a 10 ac exemption by Regulatory Division, USEPA and a Washington State Experimental Use permit (Appendix A).

### 3) Objectives

- a) At the commercial scale, and alongside the conventional carbaryl-based aerial treatment plan, compare the efficacy of imidacloprid against burrowing shrimp to the carbaryl (Sevin 80S; Bayer Corp) standard and untreated checks according to three primary variables:
  - (1) formulation of imidacloprid:
    - i) Nuprid 2F (liquid)
    - ii) Mallet 0.5G; NuFarm, Inc. (granular)
  - (2) rate of imidacloprid
    - i) 2.0 lb a.i./ac
    - ii) 1.0 lb a.i./ac
    - iii) 0.5 lb a.i./ac
  - (3) vegetation type
    - i) thick eelgrass or algal mats
    - ii) moderate densities
    - iii) bare ground
- b) In smaller (<0.1 ac) plots, compare efficacy of the two formulations according to more combinations of these same three variables (formulation, rate, and substrate type) as well as others (bed elevation, application timing, and presence of oyster seed)
- 4) On and near sites of an isolated large aerial imidacloprid treatment (10 ac of Nuprid 2F @ 2.0 lb a.i./ac), assess impact to non-target organisms:
  - (1) salmonids (e.g., juvenile Chinook and cutthroat trout)
  - (2) other fish
  - (3) Dungeness crab
  - (4) benthic infauna
- b) At that same isolated site, and selected sites of granular treatment, make preliminary assessments of imidacloprid off-bed transport in the water column and dissipation in sediments.



**Explanation and Justification of Quantity**

These trials will require a maximum of 130 lb a.i. of imidacloprid to be applied to a total of 100 total acres in Willapa Bay or Grays Harbor (Table 25). However, depending on the results of trials currently in progress, the density and distribution of burrowing shrimp next year, and the treatment schedule for the conventional carabaryl-based management program for burrowing shrimp, the actual treated acreage could be considerably lower (e.g., 70 ac). The requested acreage is required to complete the studies required for imidacloprid registration and permitting in the third of a multi-year experimental program (see point 6 below). Amounts were derived according to an

experimental design that strives for suitable replication but is constrained by limited space, time, and considerations for potential non-target impact. Our most common plot size (5 ac) tend to the low size of most commercial beds ( $\geq 10$  ac) but are still large enough to include some variation in burrowing shrimp density, substrate, vegetation, bed elevation, and drainage pattern that accompany commercial shellfish beds and impact efficacy.

Table 25. Maximum acreage and quantity of imidacloprid proposed for experimental application in Willapa Bay in 2010 according to formulation (Nuprid 2.F (liquid) or Mallet 0.5G (granular), and rate.

Material	Rate (lb a.i./ac)	No. of Beds @ Size per Bed (ac)	Acreage	lb a.i.
Nuprid 2F	2.0	4 @ 5 ac; 1 @ 10 ac*	30.0	60.0
Nuprid 2F	1.0	4 @ 5 ac	30.0	30.0
Nuprid 2F	0.5	4 @ 5 ac	20.0	10.0
Total Nuprid			70-80*	80-100
Mallet	1.0	4 @ 2.5 ac; 1 @ 10 ac	20.0	20.0
	0.5	4 @ 2.5 ac	10.0	5.0
Total Mallet			20-30*	15-25.0
Total imidacloprid			100	120-130

\* Only a single 10 ac bed will be treated; formulation will depend on results of 2009 trials.

**5) Duration**

We request that a federal experimental use permit for imidacloprid on Washington state shellfish grounds be granted for one year with anticipated renewals for at least the two following years.

We have prioritized and timed studies according to a two year registration and four year permitting process for completion in 2012. The figure shows activities planned primarily for 2008 and, to a lesser degree, 2009 and 2010. The results of studies conducted in 2009 and 2010 will determine what studies will be conducted in 2012. These include the completion of the registration process and a major modification of the current NPDES permit to include imidacloprid, which will be renewed in July 2011. As noted above, the requested acreage will likely change from year to year as well. A more complete and precise timeline for the registration of imidacloprid on Washington state shellfish grounds cannot be constructed at this time. There is little precedent for an aquatic use for this compound, so federal and state requirements have yet to fully specified.

**6) Disposition of unused material**

Almost all imidacloprid will be used during experimental application, as the amount of material applied will be precisely measured and applied using calibrated equipment. Unused material will be stored temporarily in an EPA and OSHA compliant pesticide storage unit located at the Washington State University Research and Extension Unit in Long Beach, WA. Unused material will ultimately be disposed through the Washington Department of Agriculture's Pesticide Disposal Program.



## Appendix A: Amended Study Plan for Experimental Use of Imidacloprid in Willapa Bay, 2009

Imidacloprid will be applied in association with a Washington State Experimental Use Permit to be granted in association a 10 ac exemption for experimental use of imidacloprid from a Federal EUP as documented in a forthcoming letter from Registration Division, USEPA. The Proposed Experimental Labels for granular formulations of imidacloprid are presented in Appendix C. Contact persons are Steve Booth, WGHOGA (360-867-4163, [boothswa@comcast.net](mailto:boothswa@comcast.net)) and Kim Patten, WSU (360-642-2031, [pattenk@wsu.edu](mailto:pattenk@wsu.edu)).

### 7) Locations, acreage to be treated

The primary study site is a 9 ac oyster bed located on the east bank of the Cedar River in north Willapa Bay (Figure 1). The bed, A43 (locator point = N46°43.4241' W123°57.8314'), has lain fallow for at least 20 years, yet is well drained and can be returned to a productive state if burrowing shrimp are sufficiently suppressed. Shrimp are fairly uniformly distributed across the bed and densities of 20 – 25 burrows per m<sup>2</sup>. The western fourth of the bed is densely covered with the native eelgrass, *Zostera marina*, that transitions to the bare mud/sand substrate of the rest of the bed. The two different vegetational covers comprise experimental variables that hypothetically affect the efficacy of imidacloprid. The study plot will be set at least 100 ft from the channel of the Cedar River and a major drainage channel to the north. If control is satisfactory the bed will be planted with oyster seed, but no oysters will be harvested from the bed for at least one but probably several years.

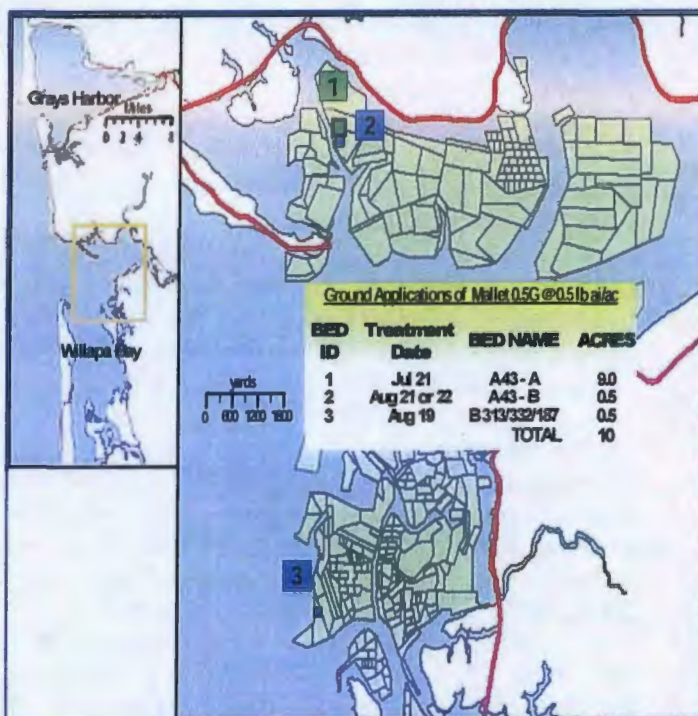


Figure 12 Name and location of 9 ac fallow oyster bed (A43) near the Cedar River in Willapa Bay to be treated with imidacloprid on July 21, 2009 (1). An additional 10<sup>th</sup> acre will be applied as two separate half-acre plots (2, 3) on August 20 or 21.

Two separate half-acre plots will comprise and additional 10<sup>th</sup> ac to be treated on August 20 or 21, 1 month after the 9 ac application. One plot is located near the 9 ac plot and the other is located further south in another area of high density burrowing shrimp which differs slightly in substrate and vegetation.

### 8) Details of the Proposed Program

A 0.5% active ingredient (a.i.) granular formulation of imidacloprid (Mallet 0.5G; NuFarm Americas) will be applied by hand using conventional granular pesticide applicators (belly grinders) at a rate of 0.5 lb a.i./ac. For the 9 ac trial, application will be during the maximum low morning tide on or about July 21, 2009. The date is comparatively close to July 22 aerial applications of carbaryl (Sevin 80SP; Bayer Corp.) to other oyster beds in Willapa Bay as part of our conventional burrowing shrimp management program. All beds treated with carbaryl lie several miles from A43.



Trials and assessments of efficacy will be directed primarily by Dr. Kim Patten, Long Beach Research Unit, Washington State University and Dr. Steven Booth, Pacific Shellfish Institute. For both small plot and commercial scale trials, efficacy will be judged primarily by comparing shrimp burrow counts taken before treatment and at several post treatment intervals (~4 – 8 weeks and, pending results, 11 months after treatment). Burrowing shrimp densities will be measured on the bed at 2 and 4 weeks prior to treatment and at 2, 4, and 8 weeks after treatment to be treated within 2 weeks prior to and at 4 - 8 weeks after treatment by counting burrows inside a 1m<sup>2</sup> grid along transects that diagonally cross the bed or otherwise adequately represent the bed. Percentage cover of eelgrass, algae, shell, and standing water will also be recorded.

Non-target field impact on the benthic in-fauna will be addressed by the Pacific Shellfish Institute, using sampling protocols which were approved by the Washington State Department of Ecology for an assessment of the Sediment Impact Zone associated with the carbaryl-based burrowing shrimp management program. Three core-replicates cores will be taken at each of 4 sites near the corners of the 9 ac study site. Cores will be taken using a PVC clam gun. Similar samples will be taken in untreated areas of the bed located several hundred meters away. Cores will be taken at 2 weeks prior to treatment and 1 month post treatment. Each core (15 cm deep x 10.2 cm in diameter) will be immediately sieved through 0.5 mm mesh using salt water and stored in a 10% buffered formalin solution for 2 weeks, then stained with rose bengal and re-sieved through 250 um mesh to remove excess detritus and stored in 70% ethanol. Polychaete identification and enumeration will be to species by Dr. Eugene, Ruff Wormworks, Inc., Puyallup, WA. Identification and enumeration of other invertebrates will be conducted by personnel at PSI. Species attributes (type and abundance) of key benthic invertebrates, as well as community descriptors (Abundance, Species Richness, and Simpson Diversity) will be used to compare treatment affects.

Non-target and sub-lethal effects on salmonids (i.e., juvenile chinook and cutthroat trout) will figure heavily in both the federal registration and state permitting of imidacloprid. A biomarker, based on imidacloprid residues in brain tissues, was successfully tested by Dr. Christian Grue, University of Washington, to address these effects. The biomarker showed good correlation between residues, created from precisely controlled exposures of chinook smolt to a range of imidacloprid concentration, to selected physiological functions (gill ATPase activity) or non-function (mortality), and overt behavioral effects (lethargy, erratic swimming, on-bottom gilling). These findings will be validated this year.

An ancillary study continues last years' tests on the utility of existing ELIZA test kits for imidacloprid residues in brain tissues. Last years' results showed high correlation among a range of imidacloprid residue concentrations identified in the brains of cutthroat trout using the ELIZA kit and standard laboratory methods.

We shall also begin preliminary assessments of the impact of the imidacloprid applications on crab populations. These will include observations of juvenile crab (20 mm carapace width) caged on treated beds, and 24 and 48 hr post bed inspections for dead or crab in tetanus shock.

Concentrations of imidacloprid in the water column will be taken preliminary to more thorough fate & transport study that will be conducted in subsequent years. Imidacloprid will be sampled and measured in water over the 9 ac bed, in off-bed channels, and in bed sediment. Two samples will be taken near each of the four corners of the bed at 1 and 6 hr after treatment and at a single location over the bed at 31 hr after treatment. Additional water samples will be taken at at least two sites in the Cedar River channel near the bed at in association with the salmonid studies. Channel water samples will be taken

at 6, 24, 31, 50, 58, 74, and 80 hr post treatment. At least two samples will be taken per sample time/site.

Concentrations of imidacloprid in sediments will be measured in core samples 5.2 cm in diameter and 10 cm deep. Three cores per sample will be composited, homogenized, and standing water will be decanted. Percent moisture will be determined before analysis and Felsot and Rupert's (2002) coefficients will be used to calculate concentrations lost to pore water. Samples will be collected prior to application, at 1 day and two weeks after application.

### Objectives

- a) Test the efficacy of granular imidacloprid at an experimental application rate of 0.5 lb a.i./ac to suppress burrowing shrimp at a large scale (9 ac) in sub-sections with
  - (1) no vegetation
  - (2) sparse vegetation
  - (3) dense vegetation
- b) On and near the 9 ac site, assess impact to non-target organisms:
  - (1) salmonids (e.g., juvenile Chinook and cutthroat trout)
  - (2) other fish
  - (3) Dungeness crab
  - (4) benthic infauna
- c) At that same isolated site, and selected sites of granular treatment, make preliminary assessments of imidacloprid off-bed transport in the water column and dissipation in sediments
- d) In smaller (<0.1 ac) plots, compare efficacy of the two formulations according to more combinations of these same three variables (formulation, rate, and substrate type) as well as others (bed elevation, application timing, and presence of oyster seed)

### 9) References

Felsot, A.S. and J.R. Rupert. 2002. Imidacloprid residues in Willapa Bay (Washington State) water and sediment following application for control of burrowing shrimp. *J. Agric. Food Chem.* 50: 4417-4423.

### 10) Disposition of unused material

Almost all imidacloprid will be used during experimental application, as the amount of material applied will be precisely measured and applied using calibrated equipment. Unused material will be stored temporarily in an EPA and OSHA compliant pesticide storage unit located at the Washington State University Research and Extension Unit in Long Beach, WA. Material will eventually be disposed through the Washington Department of Agriculture's Pesticide Disposal Program.



**21-Day Screen Completed by**  
**Contractor**

*6 months*

**21-Day Expires on** 7-22-09

**Jacket #** 86414-EUP-R  
**MRID#** \_\_\_\_\_

**Content Screen: Recommended to**  
**Pass/Fail**

**86-5 Review: Passed/Failed/****NA**

**Transfer This Jacket to:**

LINDA ARRINGTON

# PRIA 2 – 21 Day Content Screen Review Worksheet

(EPA/OPP Use Only)

3/23/09

21 Day Screen Start Date: 7-1-09

Experts In-Processing Signature: MF HARRINGTON Date 7-7-09

Fee Paid: Yes ☒

Division management contacted on issues No ☐ Yes ☐ Date \_\_\_\_\_

EPA Reg. Number: <u>86414-EUP-R</u>		EPA Receipt Date: <u>7-1-09</u>												
Items for Review				Yes	No	N/A*								
1	<b>Application Form</b> (EPA Form 8570-1)(link to form) signed & complete including package type					X								
2	<b>Confidential Statement of Formula</b> all boxes completed, form signed, and dated (EPA Form 8570-4) (Link to form) a) All inerts (link to <a href="http://www.epa.gov/opprd001/inerts/">http://www.epa.gov/opprd001/inerts/</a> ), including fragrances, approved for the proposed uses (see Footnote A) <table border="1" style="float: right; margin-top: 5px;"> <tr> <td style="width: 50px;">yes</td> <td style="width: 50px;">no</td> </tr> <tr> <td style="height: 20px;"></td> <td style="height: 20px;"></td> </tr> </table>			yes	no					X				
yes	no													
3	<b>Certification with Respect to Citation of Data</b> (EPA Form 8570-34) (Link to form) completed and signed (N/A if 100% repack)					X								
	Certificate and data matrix consistent					X								
	If applicant is relying on data that are compensable, is the offer to pay statement included. (see Footnote B) <table border="1" style="float: right; margin-top: 5px;"> <tr> <td style="width: 50px;">yes</td> <td style="width: 50px;">no</td> </tr> <tr> <td style="height: 20px;"></td> <td style="height: 20px;"></td> </tr> </table>			yes	no									
yes	no													
	If applicable, is there a letter of Authorization for exclusive use only.													
4	<b>Formulator's Exemption Statement</b> (EPA Form 8570-27) (Link to form) completed and signed (N/A if source is unregistered or applicant owns the technical)					X								
5	<b>Data Matrix</b> (EPA Form 8570-35) (Link to form) both internal and external copies (PR 98-5) (Link to PR 98-5) completed and signed (N/A if 100% repack) a) Selective Method (Fee category experts use) b) Cite-All (Fee category experts use) c) Applicant owns all data (Fee category experts use) <table border="1" style="float: right; margin-top: 5px;"> <tr> <td style="width: 50px;">yes</td> <td style="width: 50px;">no</td> </tr> <tr> <td style="height: 20px;"></td> <td style="height: 20px;"></td> </tr> <tr> <td style="height: 20px;"></td> <td style="height: 20px;"></td> </tr> <tr> <td style="height: 20px;"></td> <td style="height: 20px;"></td> </tr> </table>			yes	no									X
yes	no													
6	<b>5 Copies of Label</b> (link to <a href="http://www.epa.gov/oppfead1/labeling/lrm/">http://www.epa.gov/oppfead1/labeling/lrm/</a> ) (Electronic labels on CD are encouraged and guidance is available)( link to <a href="http://www.epa.gov/pesticides/regulating/registering/submissions/index.htm#labels">http://www.epa.gov/pesticides/regulating/registering/submissions/index.htm#labels</a> )			X										



7	Is the data package consistent with PR Notice 86-5 (link to PRN 86-5)			X
8	Notice of Filing (link to <a href="http://www.epa.gov/pesticides/regulating/tolerance_petitions.htm">http://www.epa.gov/pesticides/regulating/tolerance_petitions.htm</a> ) included with petitions (link to <a href="http://www.epa.gov/pesticides/regulating/tolerances.htm">http://www.epa.gov/pesticides/regulating/tolerances.htm</a> )			X
9	If applicable for conventional applications, reduced risk rationale (link to <a href="http://www.epa.gov/opprd001/workplan/reducedrisk.html">http://www.epa.gov/opprd001/workplan/reducedrisk.html</a> )			X
10	Required Data (link to <a href="http://www.epa.gov/pesticides/regulating/data_requirements.htm">http://www.epa.gov/pesticides/regulating/data_requirements.htm</a> ) and/or data waivers. See Footnote C.			
	a) List study (or studies) not included with application			

**Comments:**

\* There are no studies associated with this submission

AB

\* N/A – Not Applicable

**Footnotes**

A. During the 21 day initial content review, all CSFs will be reviewed to determine whether all inerts listed, including fragrances, are approved for the proposed uses. If an unapproved inert is identified, the applicant must either 1) resolve the inert issue by, for example, removing the inert, substituting it with an approved inert, submitting documentation that EPA approved the inert for the proposed pesticidal uses, correcting mistakes on the CSF, etc. or 2) provide the data to support OPP approval of the inert or 3) withdraw the application. Removing or substituting an inert ingredient will require a new CSF and may require submission of data. All information, forms, data and documentation resolving the inert issue must have been received by the Agency or the application withdrawn within the 21 day period, otherwise, the Agency will reject the application as described below.

To successfully complete this aspect of the 21 day initial content screen, applicants are **strongly encouraged** to verify that all inert ingredients have been approved for the application's uses **even if a product is currently registered** by consulting the inert Web



site [link to <http://www.epa.gov/opprd001/inerts/lists.html>] and if the inert is not approved, to **obtain the necessary inert approval prior to submitting an application to register a pesticide product containing that inert ingredient**. Some inert ingredients are no longer approved for food uses or certain types of uses. The name and/or CAS number on a CSF must match the name and CAS number on this web site. Simple typographical errors in the name or CAS number have resulted in processing delays.

If an inert is not listed on the inert ingredient web site and the applicant believes that the inert has been approved, the applicant should contact the Inert Ingredient Assessment Branch (IIAB) at [inertsbranch@epa.gov](mailto:inertsbranch@epa.gov) and resolve the issue. Copies of the correspondence with IIAB resolving the issue should accompany the application. All new inerts except PIP inerts are reviewed by IIAB. The IIAB should also be contacted for any questions on what supporting data needs to be submitted for and the Agency's inert review process. Questions on PIP inerts should be directed to the Chief of Microbial Pesticides Branch [Link to [http://www.epa.gov/oppbppd1/biopesticides/contacts\\_bppd.htm](http://www.epa.gov/oppbppd1/biopesticides/contacts_bppd.htm)].

When a brand, trade, or proprietary name of an inert ingredient is listed on a CSF, additional information such as an alternate name of the inert, CAS number or other information [link to <http://www.epa.gov/opprd001/inerts/tips.pdf>] must also be included to enable the Agency to determine if it has been approved. Each component of an inert mixture (including a fragrance) must be identified. In some cases, the supplier of the mixture or fragrance may need to provide this information to the Agency. Prior to the Agency's receipt of an application, applicants must arrange with a proprietary mixture or fragrance supplier to provide the component information to the Agency or promptly upon EPA's request. If the inert ingredients in a proprietary blend (including fragrances) cannot or are not identified or provided within the 21-day content review period, the Agency will reject the application.

During the 21 day content review, applicants should submit information to the individual identified by the Agency when the applicant is informed of an unapproved inert.

### **Unapproved Inerts Identified on CSFs**

#### **All applications except conventional new products and PIPs**

Once an unapproved inert is identified on a CSF, the Agency will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the inert's identity or CAS number, providing documentation that the inert has been approved, or removing the unapproved inert from the CSF or replacing it with one that is approved for the application's uses; or
2. Submit the information and data needed for the Agency to approve the unapproved inert. If this option is selected and implemented, the Agency may request an extension in the PRIA decision review timeframe to accommodate the inert review/approval process;

3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of these options is selected and implemented by the applicant within the 21 day content review period, the Agency will reject the application and retain 25% of the full fee of the category identified.

#### Conventional New Product Applications

When the Registration Division identifies an unapproved inert on a CSF with an application for a new product that the applicant has not identified as requiring an inert approval (R311, R312 or R313), it will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the inert's identity or CAS number, providing documentation that the inert has been approved, or removing the unapproved inert from the CSF or replacing it with one that is approved for the application's uses; or
2. Submit the information and data needed for the Agency to approve the unapproved inert, including any required petition to establish or amend a tolerance or exemption from a tolerance. (This option may change the PRIA category for the application, which could require a longer decision review time and a larger fee. If additional fees are due, they must be received by the Agency within the 21 day content review period.)
3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of the above options is selected and implemented during the 21-day content-review period, the Agency will reject the application and retain 25% of the appropriate fee for the new product-inert approval category.

#### PIP Applications

When the Biopesticide and Pollution Prevention Division identifies an unapproved inert on a PIP CSF and a request to approve the inert does not accompany the application, it will contact the applicant with the following options:

1. Correct the application by, for instance, correcting the spelling or name of the inert to that in 40 CFR 174, or providing documentation that the inert has been approved; or
2. Submit the information and data needed for the Agency to approve the unapproved inert. If an inert ingredient tolerance exemption petition is required, the petition must be received by the Agency and the B903 fee paid within the 21 day period. If this option is selected and implemented, the Agency will discuss harmonizing the timeframe for both actions.



3. Withdraw the application (the Agency retains 25% of the full fee for the fee category estimated); or

If none of the above options is selected and implemented during the 21 day content review period, the Agency will reject the application and retain 25% of the fee.

B. A policy on documentation of offers to pay is still being developed, however, for a me-too or fast track (similar/identical) new product, R300 or A530, an application without the necessary authorizations of offers to pay will be placed into either R301 or A531. The Agency recommends that authorizations of offers to pay be submitted with other PRIA applications to avoid delays in the Agency's decision.

C. Biopesticide applicants are advised to contact the Agency and discuss study waivers prior to submitting their application to the Agency. Documentation of such discussions should be submitted with the study waiver.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

July 6, 2009

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

OPP Decision Number: D-416578  
EPA File Symbol or Registration Number: 86414-EUP-R  
Product Name: Imidacloprid against burrowing shrimp on Willapa Bay and Grays Harbor shellfish beds  
EPA Receipt Date: 01-Jul-2009  
EPA Company Number: 86414  
Company Name: WASHINGTON STATE UNIVERSITY

KIM PATTEN  
WASHINGTON STATE UNIVERSITY  
CAHNRS AGRICULTURAL RESEARCH CENTER  
HULBERT 403  
PULLMAN, WA 99164-6240

SUBJECT: Receipt of EUP Application and 100% State/Federal Waiver Request

Dear Registrant:

The Office of Pesticide Programs has received your EUP application for registration and 100% state/federal waiver request. If you submitted data with this application, the results of the PRN-86-5 screen will be communicated separately. During the administrative screen, the Office of Pesticide Programs has determined that this Action is subject to a Pesticide Registration Service Fee as defined in the Pesticide Registration Improvement Act.

The Action has been identified as Action Code: R250


NEW USE;OUTDOOR;NON-FOOD;WITH EXPERIMENTAL USE PERMIT (NO CREDIT TOWARD NEW USE REGISTRATION);

Your request for waiver has been forwarded for review. You will be notified in writing when a determination is made regarding your request. If the determination indicates that payment is due, you will receive instructions for submitting payment at that time.



If you have any questions, please contact the Pesticide Registration Service Fee Ombudsman, at (703) 305-6249.

Sincerely,

A handwritten signature in cursive script that reads "Teresa Downs".

Front End Processing Staff

Information Technology & Resources Management Division

# Willapa-Grays Harbor Oyster Growers Association

P.O. Box 3 Ocean Park, WA 98640

**From:**

Steven R. Booth, Ph.D.  
IPM Coordinator, WGHOGA  
Senior Scientist, Pacific Shellfish Institute  
2711 44<sup>th</sup> Ave. N.W.  
Olympia, WA 98502  
360-867-4163  
[boothswa@comcast.net](mailto:boothswa@comcast.net)  
[booths@pacshell.org](mailto:booths@pacshell.org)

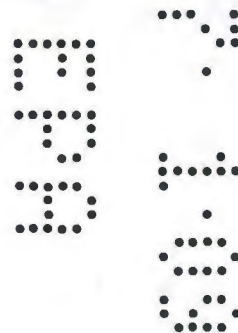
Tim Morris  
President, WGHOGA  
P.O. Box 3  
Ocean Park, WA 98640

Dr. Kim Patten  
Extension Specialist, Professor  
Washington State University  
Long Beach Research Unit  
2907 Pioneer Road  
Long Beach WA 98631  
360-642-2031  
Mobile Phone 360-355-7864  
[pattenk@wsu.edu](mailto:pattenk@wsu.edu)

**To:**

Meredith Laws, Branch Chief  
Insecticide-Rodenticide Branch  
Registration Division  
USEPA

John Hebert, PM 7 USEPA  
Insecticide-Rodenticide Branch  
Registration Division  
Rom S-4900  
One Potomac Yard  
2777 South Crystal Drive  
Arlington, VA 22202-4501



June 30, 2009

**RE:** Application for Federal Experimental Use Permit for use of imidacloprid against burrowing shrimp on Willapa Bay and Grays Harbor shellfish beds.

Dear Drs. Laws and Hebert:

Please find attached our application for a second annual Federal Experimental Use Permit to experimentally treat 67.5 ac of intertidal shellfish beds in Willapa Bay with imidacloprid to suppress local infestations of pestiferous burrowing shrimp. As discussed in greater detail below, and in the attached application, we are applying for a higher rate of the liquid imidacloprid formulation than under our previous FEUP, and for additional experimental acreage for treatment with a granular formulation of imidacloprid. These approaches could provide sufficient efficacy against burrowing shrimp at the commercial scale, in contrast to last year's trials, which demonstrated generally poor results.

The proposed experimental use is part of on-going efforts towards a 3C registration of imidacloprid for this use by the Willapa Bay Grays Harbor Oyster Growers Association (WGHOGA) and is in collaboration with NuFarm Americas. The registration effort, in turn, is part of a larger effort to develop and implement a comprehensive IPM program for burrowing shrimp on commercial shellfish beds. Although the current primary management tool, aerial applications of carbaryl (Sevin® 80SP, Bayer Corp.) has consistently demonstrated sufficient efficacy with minimal and transitory non-target impact, a variety of groups continue to challenge the use of many conventional pesticides (i.e., organophosphate and carbamate) in a variety of crops, including our use. In recent years, we have investigated dozens of alternative



management tactics for burrowing shrimp and continue to examine the very few with demonstrated potential with hopes of replacing carbaryl by 2012. This leaves the shellfish industry with a very limited amount of time for full implementation. At this time, imidacloprid is the only alternative approach with high potential to adequately suppress burrowing shrimp with minimal impact to non-target organisms that also has enough corporate support to request for third party registration.

So far, the maximum rate for imidacloprid on terrestrial crops has been 0.5 lb a.i./ac, as Terrestrial Field Dissipation Studies conducted by the original registrant (Bayer Corp.) were at that rate. Preliminary small plot trials (<0.1 lb a.i./ac) demonstrated imidacloprid (Admire 2EC) to be comparably effective at that rate as carbaryl (Sevin 80WP or Sevin 80SP) was at 10 lb a.i./ac. Accordingly, we conducted last years' large scale commercial trials of Nuprid 2F, (FEUP #d390549) using an experimental rate 0.5 lb a.i./ac. Results showed generally poor efficacy, likely as a result of heavy vegetative cover, greater tidal runoff, and other factors that due not always occur in the very small plot (<0.1 ac) trials allowable under Washington State Experimental Use Permits. A higher rate of the liquid imidacloprid formulation (Nuprid 2F) or the substitution of the liquid with a granular formulation (Mallet 0.5G, NuFarm Americas) could be provide sufficient efficacy against burrowing shrimp at the commercial scale. Preliminary small plot trials this spring have supported that hypothesis (Effectiveness Data, Table 23 & 24, Attachment 2).

The objective of Bayer's Terrestrial Field Dissipation Studies was to address potential transport of imidacloprid into ground water and subsequently into wells and the drinking water supply. The primary concern was to human health. Those trials were particularly critical to imidacloprid in field crops, where it is often applied as a seed coating to suppress subterranean insect pests, thus its mode of entry into the ground water could theoretically be facilitated. Our applications of imidacloprid to limited acreage in Willapa Bay will not leach into ground water, nor will it have any opportunity to enter any reservoir of drinking water. It will likely quickly dissipate into the hundreds of thousands of gallons of moving waters within the estuary.

Under the proposed FEUP, we wish to apply imidacloprid at a rate higher than 0.5 lb a.i./ac to only 35 of the total 67.5 acres for which we are applying (20 ac @ 2.0 lb a.i./ac and 15 ac @ 1 lb a.i./ac) (see Justification and Explanation of Quantity, Attachment 2). In addition, we plan to preliminarily examine the fate and transport of imidacloprid in association with the studies proposed here (Details of the Proposed Program, Attachment 2). We have initiated dialogue with the EPA, IR-4, and NuFarm to consider allowing a 3C registration by the WGHOGA of liquid imidacloprid for this use at 2.0 lb a.i./ac and to understand what additional steps, if any, should be taken, for such a registration. Both IR-4 and NuFarm support this approach.

We have already assembled and derived much of the data relevant to the proposed use, including impact to non-target invertebrates and fish, especially salmonids, as well as efficacy data (see Attachment 1). Almost all preliminary data showed imidacloprid (Admire 2EC, Bayer Corp. or Nuprid 2F, NuFarm Americas) to be comparably effective as the standard pesticide for this use, carbaryl (Sevin 80SP, Bayer Corp) with lower potential for non-target impact.

As noted in the study plan, the proposed experimental applications of imidacloprid occur in

tandem with conventional aerial applications of Sevin on selected shellfish parcels in Willapa Bay and Grays Harbor. This year's conventional program features applications of Sevin to 560 ac distributed among 44 beds. The proposed experimental imidacloprid treatments have been carefully planned in coordination with that program. Carbaryl applications are scheduled to begin on July 7 - 9 in areas where we do not plan to use imidacloprid. More applications will occur during the maximum low tides of the next tidal interval, which occurs July 21 - 23. The imidacloprid treatments are scheduled to occur on the 21, before the carbaryl applications to avoid potential cross-contamination. (Cross contamination is also avoided by placing beds targeted for carbaryl treatments at great distance from the experimental beds, or if close they are close, on the earlier tidal interval).

We realize that these dates are very rapidly approaching. Unfortunately, the development of both the experimental plan and the conventional plan for burrowing shrimp management were greatly delayed by the unseasonably cool spring which suppressed shrimp activity, as well as the normal difficulties in accessing shellfish beds during the limited number of hours at daylight low tides that occur in the spring.

Accordingly, we respectfully ask that this application be processed as quickly as possible. The loss of this years data could severely damage the prospects of our goal of imidacloprid registration by 2012. If there is anything we could do to facilitate the process, please let us know.

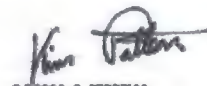
Sincerely,



Steven R. Booth



Tim Morris





Receipt for Experimental Use Permit			
S:	853268	Resubmission:	<input type="radio"/> Yes <input checked="" type="radio"/> No
Regulatory Type:	Experimental Use Permit - Section 5	Fee For Service:	<input checked="" type="radio"/> Yes <input type="radio"/> No
Application Type:	New Registration	Billable:	<input checked="" type="radio"/> Yes <input type="radio"/> No
Company:	86414 WASHINGTON STATE UNIVERSITY	V	
Risk Mgr:	Registration Division, Risk Management Team 7		
EUP #:	86414-EUP-R	Crop Destruction:	<input type="radio"/> Yes <input checked="" type="radio"/> No
Override #:		BioTech Notification Exemption Petition:	<input type="radio"/> Yes <input checked="" type="radio"/> No
Parent Section3:		BioTechnology Notification:	<input type="radio"/> Yes <input checked="" type="radio"/> No
Parent Product Name:			
Application Date:	16-Jun-2009	OPP Rec'd Date:	01-Jul-2009
Front End Date:	06-Jul-2009	Risk Manager Send Date:	06-Jul-2009
FFS Due Date:	07-Jan-2010	Negotiated Due Date:	
OPP Target Date:			
Fast Track:	<input type="checkbox"/>	New Ingredient:	<input type="checkbox"/>
Receipt Description:		View/Edit	
imidacloprid against burrowing shrimp on Willapa Bay and Grays Harbor shellfish beds: associated with EPA reg. no. 228-484 (Nuprid 2F)		New Ingredient Request Date: <input type="text"/>	
		New Ingredient Received Date: <input type="text"/>	
Form A:	<input type="checkbox"/>	Signature Date:	<input type="text"/>
Form B:	<input type="checkbox"/>	Signature Date:	<input type="text"/>

Print Letter

Enter More Information

Tracking

Receipt Content	Des
Paper Label	

# Fee for Service

853143C~

This package includes the following

☒ New Registration

☐ Amendment

☐ Studies? ☒ Fee Waiver?

☐ volpay % Reduction: 100%

for Division

☐ AD

☐ BPPD

☒ RD

Risk Mgr.

7

Receipt No.

S-

~~853143~~ 853266

EPA File Symbol/Reg. No.

~~86414~~  
~~81959~~ EUP-R

Pin-Punch Date:

7/1/2009

☐ This item is NOT subject to FFS action.

## Action Code:

Requested:

None

Granted:

R250

Amount Due: \$ 17,136

100% fee waiver granted.

## Parent/Child Decisions:

☒ Inert Cleared for Intended Use

☐ Uncleared Inert in Product

Reviewer: RKumar

Date: 7-6-09

Remarks: Note to JJ: The applicant is from Washington State University, so 100% fee waiver was granted. Contact John Hebert if you have any questions.  
Note to John H: Very short review time - 2 weeks.





UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

July 6, 2009

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

KIM PATTEN  
WASHINGTON STATE UNIVERSITY  
CAHNRS AGRICULTURAL RESEARCH CENTER  
HULBERT 403  
PULLMAN, WA 99164-6240

Subject: Assignment of New EPA Company Number

Dear Registrant:

The Office of Pesticide Programs received your request for a company/distributor number. The company number assigned to you is 86414.

You are required to notify the Agency of any change in name or address. All requests for change of company name and/or address, appointment of agent or withdrawal of an agent's appointment, must be sent to the following address:

Document Processing Desk (COADR)  
Office of Pesticide Programs (7504P)  
U.S. Environmental Protection Agency  
1200 Pennsylvania Avenue, N.W.  
Washington, DC 20460

All products must be registered with the Agency prior to shipment and/or sale. Information on registering pesticide products can be obtained by calling the Registration Division Ombudsperson at (703) 308-8893. Requests for a Pesticide Registration Kit can be obtained via e-mail to: [Pearlman.Michael@epa.gov](mailto:Pearlman.Michael@epa.gov). If you are only distributing a product you must complete the Notice of Supplemental Registration of Distributor (EPA form 8570-5). This form can also be obtained by calling the number listed above or can be downloaded at <http://www.epa.gov/opprd001/forms>.

Sincerely,  
Front End Processing Staff  
Information Services Branch  
Information Technology & Resources Management Division

Form Approved. OMB No. 2070-0040.



United States  
ENVIRONMENTAL PROTECTION AGENCY  
Washington, DC 20460

OPP Identifier Number

Office of Pesticides Programs (7505C)

**Application for Experimental Use Permit to Ship and  
Use a Pesticide for Experimental Purposes Only**

**1. Type of Application**



New



Amendment (See No. 2)



Extension (Give Permit Number below)

Permit Number

**2. Briefly explain (attach a separate sheet if necessary)**

This EUP is to be used to investigate the efficacy and nont-target effects of imidacloprid against burrowing shrimp in Willapa Bay and Grays Harbor, Washington.

**3. Name and Address of Firm/Person to Whom the Experimental Use Permit is to be issued (Include Zip Code) (Type or Print)**

Ralph Cavalleri, Associate Dean and Director  
CAHNRS Agricultural Research Center, Hulbert 403  
Washington State University  
Pullman, WA 99164-6240

**4. Name and Address of Shipper only if shipment is intended or if different from applicant's name and address (Include Zip Code) (Type or Print)**

Nufarm Americas Inc.  
150 Harvester Dr., Suite 200  
Burr Ridge, IL 60527

EPA Company Number 86414 - 228022 EUP-R

**5. Name of Product**

Name of registered product: Nuprid 2F

**6. Is Product Registered with EPA?**



No



Yes (Give Registration Number or File Symbol below)

Registration Number EPA Reg. No. 228-484

File Symbol \_\_\_\_\_

**7. Total Quantity of Product Proposed for Shipment/Use**

Pounds of formulated product 332

Pounds of active ingredient 80

**8. Acreage or Area to be Treated**

maximum 57.5 (20 ac@2 lb a.i./ac, 15 ac@1 lb a.i./ac, 22.5 ac@0.5 lb a.i./ac)

**9. Proposed Period of Shipment/Use**

June 2009 / June - October 2009

**10. Places from which Shipped**

Nufarm Inland Empire Dist  
1211E St Helens ST STE B, Pasco, WA 99301

**11. Crop/Site to be Treated**

Oysters and Manila Clams (Tapes philippinarum)  
Willapa Bay and Grays Harbor, Washington

**12. Specify the name and number of the contact person most familiar with this application.**

Kim Patten 360-642-2031  
Steven R. Booth 360-867-4163

**13. Signature of Applicant or Authorized Firm Representative**

*[Signature]*

**14. Title**

WBGHOGA IPM Coordinator

**15. Date Signed**

06/16/2009

**Certification**

This is to certify that food or feed derived from the experimental program will not be used or offered for consumption or sale for consumption, except by laboratory or experimental animals, if illegal residues are present in or on such food or feed.

I certify that the statements I have made on this form and all attachments thereto are true, accurate, and complete. I acknowledge that any knowingly false or misleading statement may be punishable by fine or imprisonment, or both, under applicable law.

**Show to EPA Use Only**

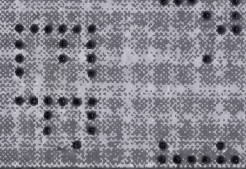
Is any correspondence to this application, refer to this number

Received by  
EPA-OPP Registration Division  
Washington, DC 20460

Normal review time indicates that processing of this application should be completed by (date)

Name of EPA Contact Person

Telephone Number





# **NUPRID 2F**

## **FOR EXPERIMENTAL USE ONLY**

Experimental Use Permit Number:

**NOT FOR SALE TO ANY PERSON OTHER THAN A PARTICIPANT IN  
THE EXPERIMENTAL USE PROGRAM**

---

Permittee:  
Ralph Cavalieri  
Associate Dean and Director  
CAHNRS Agricultural Research Center  
Hulbert 403  
Washington State University  
PO Box 646240  
Pullman, WA 99164-6240

---

**ACTIVE INGREDIENT:**

Imidacloprid: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine . . . . . 21.4%

OTHER INGREDIENTS: . . . . . 78.6%

TOTAL: . . . . . 100.0%

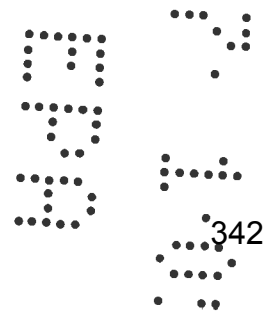
Contains 2 pounds of imidacloprid per gallon.

## **KEEP OUT OF REACH OF CHILDREN**

## **CAUTION – CAUCION**

Si usted no entiende la etiqueta, busque a alguien para que se la explique a usted en detalle.  
(If you do not understand the label, find someone to explain it to you in detail.)

EPA Permit No.



FIRST AID	
<b>If swallowed:</b>	<ul style="list-style-type: none"> <li>• Call a poison control center or doctor immediately for treatment advice.</li> <li>• Have person sip a glass of water if able to swallow.</li> <li>• Do not induce vomiting unless told to do so by the poison control center or doctor.</li> <li>• Do not give anything by mouth to an unconscious person.</li> </ul>
<b>If inhaled:</b>	<ul style="list-style-type: none"> <li>• Move person to fresh air.</li> <li>• If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible.</li> </ul>
<b>If on skin or clothing:</b>	<ul style="list-style-type: none"> <li>• Take off contaminated clothing.</li> <li>• Rinse skin immediately with plenty of water for 15-20 minutes.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>If in eyes:</b>	<ul style="list-style-type: none"> <li>• Hold eye open and rinse slowly and gently with water for 15-20 minutes, then continue rinsing eye.</li> <li>• Call a poison control center or doctor for treatment advice.</li> </ul>
<b>NOTE TO PHYSICIAN</b> No specific antidote is available. Treat the patient symptomatically.	

**PRECAUTIONARY STATEMENTS**  
**HAZARDS TO HUMANS AND DOMESTIC ANIMALS**  
**CAUTION**

Harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with skin, eyes, or clothing. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Applicators and other handlers must wear:**

- Long-sleeved shirt and long pants
  - Chemical-resistant gloves made of any waterproof material such as barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, natural rubber, polyethylene, polyvinylchloride (PVC) or viton
  - Shoes plus socks
  - Protective eyewear when working in a non-ventilated space
- Follow manufacturer's instructions for cleaning/maintaining PPE. If instructions for washables do not exist, use detergent and hot water. Keep and wash PPE separately from other laundry.

**ENGINEERING CONTROLS STATEMENTS**

When handlers use closed systems, enclosed cabs, or aircraft in a manner that meets the requirements listed in the Worker Protection Standard (WPS) for agricultural pesticides [40 CFR 170.240 (d)(4-6)], the handler PPE requirements may be reduced or modified as specified in the WPS.

**PERSONAL PROTECTIVE EQUIPMENT (PPE)**

**Users must:**

- Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet.
- Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
- Remove PPE immediately after handling this product. Wash the outside of gloves before removing.

**DIRECTIONS FOR USE**

**It is a violation of Federal law to use this product in a manner inconsistent with its labeling. A copy of this label must be in the possession of the user at the time the product is applied.**

**READ THIS LABEL:** Read the entire label and follow all use directions and precautions.

**MIXING INSTRUCTIONS:**

To prepare the application mixture, add a portion of the required amount of water to the spray tank, begin agitation, and add the Imida. Complete filling tank with the balance of water needed. Be sure to maintain agitation during both mixing and application.

**Do NOT formulate this product into other end-use products.**

**APPLICATION INSTRUCTIONS**

To test efficacy to burrowing shrimp, transport, dissipation, and non-target effects in Willapa Bay and Grays Harbor, apply at a maximum rate of 2.0 lb a.i./ac using the following properly calibrated application equipment:

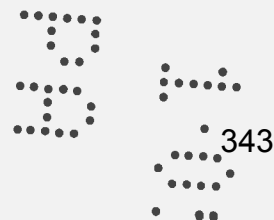
- helicopters equipped with boom 3/4 as long as rotor diameter equipped with Accu-flo™ or similar large-orificed nozzles designed for precise application.
- backpack sprayer equipped with 5' 11025 a.i. nozzle boom with a 11' pattern at 55 psi and 15 to 20 gpa depending on ground type.
- dual 10' or single 12' boom with 8002 nozzles mounted on a semi-amphibious vehicle (Argo™) at ~ 20 gpa.
- SpikeWheel™ spoke wheel subsurface injectors operated from a floating platform at ~20 gpa.

**RESTRICTIONS:**

- Do not harvest clams or oysters within one year after treatment.
- All ground must be properly staked and flagged to protect adjacent shellfish and water areas. For aerial applications, the corners of each plot marked for treatment shall be marked so the plot is visible from an altitude of at least 500ft.
- For aerial and ground-based topical applications and ground-based subsurface injection, all applications must be on beds exposed at low tide. Subsurface injections from a floating platform must be applied to beds under water.
- Aerial applications (not ground-based topical applications and subsurface injection), all applications must occur between June 1 and October 31.
- A 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray.
- Do not apply aerially during the July 4 or other holiday weekends
- During aerial applications, all public access areas within one-quarter (¼) mile and all public boat launches within a one-and-a-half (1½) mile radius of any bed scheduled for treatment shall be posted. Public access areas shall be posted at 500 foot intervals at those access areas more than 500 feet wide. Signs shall be a minimum of 8½ x 11 inches in size, and be made of a durable weather-resistant, white material. Lettering shall be in bold black type with the word "WARNING" or "CAUTION" at least one-inch high, and all other words at least one-fourth (¼) of an inch high. Signs shall also state "Do Not Fish, Crab, or Clam". Signs shall be posted so they are secure from the normal effects of weather and water currents, but cause no damage to private or public property. Signs shall be posted at least 2 days prior to treatment and shall remain for at least 3 days after treatment.

**SPRAY DRIFT MANAGEMENT**

The interaction of many equipment and weather related factors determine the potential for spray drift. Wind speed at the time of application is not to exceed 10 mph to minimize drift to adjacent shellfish and water areas. Drift potential increases at wind speeds of less than 3 mph (due to inversion potential) or more than 10 mph. However, many factors, including droplet size and canopy and equipment specifications determine drift potential at any given wind speed. Do not apply when winds are greater than 10 mph or during temperature inversions.





### Restrictions During Temperature Inversions

Because the potential for spray drift is high during temperature inversions, do NOT make ground applications during temperature inversions. Temperature inversions restrict vertical air mixing, which causes small suspended droplets to remain close to the ground and move laterally in a concentrated cloud. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. They begin to form as the sun sets and often continue into the morning. Their presence can be indicated by ground fog; however if fog is not present, inversions can also be identified by the movement of smoke from a ground source. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical mixing. The applicator is responsible for considering all of these factors when making application decisions.

### Importance of Droplet Size

An important factor influencing drift is droplet size. Small droplets (<150-200 microns) drift to a greater extent than large droplets. Within typical equipment specifications, applications are to be made to deliver the largest droplet spectrum that provides sufficient control and coverage. Formation of very small droplets may be minimized by appropriate nozzle selection.

### Mixing and Loading Requirements

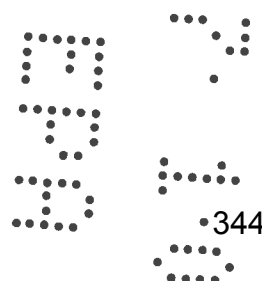
The use of a properly designed and maintained containment pad for mixing and loading of any pesticide into application equipment is recommended. If containment pad is not used, maintain a minimum distance of 25 feet between mixing and loading areas and potential surface to groundwater conduits such as field sumps, uncased well heads, sinkholes, or field drains.

### STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.

**Pesticide Storage:** Store in a cool, dry place and in such a manner as to prevent cross contamination with other pesticides, fertilizers, food, and feed. Store in original container and out of reach of children, preferably in a locked storage area. Handle and open container in a manner as to prevent spillage. If the container is leaking or material spilled for any reason or cause, carefully dam up spilled material to prevent runoff. Refer to Precautionary Statements on label for hazards associated with the handling of this material. Do not walk through spilled material. Absorb spilled material with absorbing type compounds and dispose of as directed for pesticides below. In spill or leak incidents, keep unauthorized people away.

**Container Disposal Guidance:** Pesticide containers must be properly cleaned prior to disposal. The best time to clean empty pesticide containers is during mixing and loading, because residue can be difficult to remove after it dries. Triple rinse (or pressure rinse) the pesticide container, empty all pesticide rinse water into the spray tank, and apply to a labeled crop or site. Recycling cleaned containers is the best method of container disposal. Information regarding the recycling of empty and cleaned plastic pesticide containers in Washington is available on the internet from WSU at <http://pep.wsu.edu/waste/wd.html> or from WSDA at <http://agr.wa.gov/PestFert/Pesticides/WastePesticide.htm>. Cleaned containers may also be disposed of in a sanitary landfill, if permitted by the county. Burning is not a legal method of container disposal in Washington.



**ATTACHMENT 1 – Explanation and Justification**

Two indigenous species of burrowing shrimp severely impact both the mudflat community and oyster production in Willapa Bay and Grays Harbor, WA. Both ghost shrimp (*Neotrypaea californiensis*) and mud shrimp (*Upogebia pugettensis*) reside in burrows beneath the mudflat surface, where they abrogate habitat from other benthic organisms and severely disrupt the structure of the mudflat substrate by bioturbation, causing cultured and native bivalves to sink and die. Although indigenous, both species, but particularly ghost shrimp, have greatly increased in density and distribution in the last 60 years, likely due to a combination of factors including loss of seasonal freshwater influx since the damming of the Columbia River and a decrease in key predators due to over-fishing.

Since the 1960s, applications of carbaryl (Sevin® 80SP, Bayer Corp.) on selected and legally limited acreage of commercial oyster beds, have effectively suppressed burrowing shrimp. A single application usually sufficed through multiple years of oyster development. A suite of best management practices, such as seasonal placement of carbaryl to avoid migratory salmon and pre-season monitoring of target beds, ensured that the estuarine ecosystem was not significantly affected. However, the potential impact of many conventional (i.e., organophosphate and carbamate) pesticides has been questioned by a variety of groups. This was most recently demonstrated by the National Marine Fisheries Biological Opinion regarding the impact of three carbamate pesticides on Pacific Endangered Salmon. While the final outcome of that opinion has yet to be determined, it indicates an increasingly challenging future for the use of carbaryl against burrowing shrimp in Willapa Bay and Grays Harbor.

Without the ability to manage burrowing shrimp, a significant portion of the local shellfish industry would no longer be economically viable. In 1990, oyster aquaculture accounted for one of every twelve jobs in Pacific County. Since then, the decline in marine fisheries has made the local economy even more dependent on shellfish production. As demonstrated elsewhere, the collapse of agricultural and other resource-based industries often leads to increased private development and pollution.

Efforts by the Willapa Bay / Grays Harbor Oyster Growers Association (WGHOGA) to develop an IPM program have been ongoing since the inception of the carbaryl-based program, but were formalized in 2001 when a memorandum of agreement was signed with several organizations and state agencies to develop an IPM program. Investigations of alternatives to carbaryl currently involves dozens of scientists, extension agents, and grower-collaborators who focus on biological, mechanical, and chemical controls, as well as a better understanding of burrowing shrimp ecology. Some biological control options show potential for implementation in the future, but will require much more research. Some reduced risk compounds partially suppress burrowing shrimp populations, but densities remain above farmable levels. At this point, we have identified only a single alternative tactic, imidacloprid, that has sufficient efficacy, environmental compatibility, and potential for registration to control burrowing shrimp and allow shellfish farming to continue in Southwest Washington beyond 2012.



Although preliminary very small plot trials of imidacloprid (Admire 2EC @ 0.5 lb a.i./ac) showed efficacy comparable to carbaryl (Sevin WP or SP @ 10 lb a.i./ac), the results of last years commercial large scale trials were disappointing (see Effectiveness Data, Figure 6, Attachment 2). Hypothetical reasons for the general failure in efficacy suggested that a higher rate of the liquid formulation or the substitution of the liquid with a granular formulation of imidacloprid could be provide sufficient efficacy against burrowing shrimp at the commercial scale. Preliminary small plot trials this spring have supported that hypothesis (Effectiveness Data, Tables 23, 24).

So far, the maximum rate for imidacloprid on terrestrial crops has been 0.5 lb a.i./ac, as Terrestrial Field Dissipation Studies conducted by the original registrant (Bayer Corp.) were at that rate. However, the objective of those studies was to address transport of imidacloprid into ground water and from there into wells and the drinking water supply. The primary concern was to human health. Those trials were particularly critical to imidacloprid in those systems, where it is often applied as a seed coating against subterranean insect pests, thus its mode of entry into the ground water could theoretically be facilitated.

Our applications of imidacloprid to limited acreage in Willapa Bay will not leach into ground water, nor will it have any opportunity to enter drinking water reservoirs. Imidacloprid from our treatments will quickly dissipate into the hundreds of thousands of gallons of moving waters within the estuary. Furthermore, we wish to apply imidacloprid at a rate higher than 0.5 lb a.i./ac to only 35 of the total 67.5 acres for which we are applying (20 ac @ 2.0 lb a.i./ac and 15 ac @ 1 lb a.i./ac) (see Justification and Explanation of Quantity, Attachment 2). In addition, we plan to preliminarily examine the fate and transport of imidacloprid in association with the studies proposed here (Details of the Proposed Program, Attachment 2). Additional related studies include an anaerobic metabolism study, planned to initiate very soon, and a field sediment dissipation study, planned for next year's commercial trials.

We have initiated dialogue with the EPA, IR-4, and NuFarm to consider allowing a 3C registration by the WGHOA of liquid imidacloprid for this use at 2.0 lb a.i./ac and to understand what additional steps, if any, should be taken for such a registration. Both IR-4 and NuFarm support this approach.

These attachments and forms comprise the Application for an Experimental Use Permit to Ship and Use a Pesticide for Experimental Purposes Only (8570-17) with respect to imidacloprid to manage burrowing shrimp on Willapa Bay / Grays Harbor shellfish beds. The permit will allow us to continue tests of efficacy and non-target impact at a scale that more closely approximates commercial applications. These and subsequent tests will allow imidacloprid to advance toward registration and state permitting.

**ATTACHMENT 2****A) Chemical and Physical Properties**

- 1) Chemical names: 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine,  
1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine.
- 2) Molecular formula:  $C_9H_{10}ClN_5O_2$
- 3) Tradename: Imida E-AG 2 F (EPA Reg. No. 81959-22)
- 4) Formulation (2 lbs active ingredient per gallon) of imidacloprid
- 5) CAS Number: 13826-41-3
- 6) Molecular Weight: 255.7
- 7) Water Solubility: 0.51 g/l (200° C)
- 8) Solubility in Other Solvents: @ 20° C
  - a) dichloromethane - 50.0 - 100.0 g/l
  - b) isopropanol - 1.0-2.0 g/l
  - c) toluene - 0.5-1.0 g/l
  - d) n-hexane - <0.1 g/l
  - e) fat - 0.061 g/100g
- 9) Melting Point: 136.4-143.8° C., 143.8° C (crystal form 1) 136.4° C (crystal form 2)
- 10) Vapor Pressure: 0.2 uPa (20° C) ( $1.5 \times 10^{-9}$  mmHg)
- 11) Partition Coefficient: 0.57 (22° C). (Kidd, H. and James, D. R., Eds. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, 1991 (As Updated).10-2)
- 12) Adsorption Coefficient:
  - a) in a low organic carbon silt loam (0.9% OC),  $K_d = 2.4$  mL/g (Oi, M. 1999. Time-dependent sorption of imidacloprid in two different soils. J. Agric. Food Chem. 47: 327-332.13).
  - b) see Table 1. (Felsot and Rupert, 2002).

Table 1. Sediment Distribution Coefficients ( $K_d$ ) and Freundlich Sorption Coefficient ( $K_f$ ) for Imidacloprid in Willapa Bay Sediments and Sediments Mixed with Activated Carbon.

Initial solution concn, mg/L	sediment distribution coefficient ( $K_d$ , mgL/g)		
	CaCl <sub>2</sub>	saltwater	saltwater carbon/sediment (1:2)
0.01	0.59	0.52	3912
0.1	0.62	0.52	824
1	0.51	0.45	785
10	0.39	0.32	766
100	0.28	0.24	763
av $K_d$	0.48	0.41	1410
SD	0.14	0.13	1399
$K_f$	0.46	0.40	520
1/n	0.91	0.91	0.86

**B) Proposed Label**

See separate documents



**C) Toxicity Data and Summary** [1-7 mostly from ETOXNET (<http://extoxnet.orst.edu/pips/imidaclo.htm>)]

- 1) Acute toxicity
  - a) ORL-RAT: LD<sub>50</sub> 450 mg kg<sup>-1</sup> (Meister 1994)
  - b) ORL-MUS: LD<sub>50</sub> 131 mg kg<sup>-1</sup> (Kidd and James 1991)
  - c) 24-hour DML-RAT: >5,000 mg/kg.
  - d) Non-irritating to eyes and skin (rabbits), and non-sensitizing to skin (guinea pigs) (Kidd and James 1991)
- 2) Chronic Toxicity
  - a) A 2-year feeding study in rats fed up to 1,800 ppm resulted in a No Observable Effect Level (NOEL) of 100 ppm (5.7 mg/kg body weight in males and 7.6 mg/kg in females). Adverse effects included decreased body weight gain in females at 300 ppm, and increased thyroid lesions in males at 300 ppm and females at 900 ppm.
  - b) A 1-year feeding study in dogs fed up to 2,500 ppm resulted in a NOEL of 1,250 ppm (41 mg/kg). Adverse effects included increased cholesterol levels in the blood, and some stress to the liver (measured by elevated liver cytochrome p-450 levels) (Federal Register 1995).
- 3) Reproductive Effects
  - a) A three generation reproduction study in rats fed up to 700 ppm imidacloprid resulted in a NOEL of 100 ppm (equivalent to 8 mg/kg/day) based on decreased pup body weight observed at the 250 ppm dose level (Federal Register 1995).
- 4) Teratogenic Effects
  - a) A developmental toxicity study in rats given doses up to 100 ppm by gavage on days 6 to 16 of gestation resulted in a NOEL of 30 mg/kg/day (based on skeletal abnormalities observed at the next highest dose tested of 100 ppm) (Federal Register 1995)
  - b) In a developmental toxicity study with rabbits given doses of imidacloprid by gavage during days 6 through 19 of gestation, resulted in a NOEL of 24 mg/kg/day based on decreased body weight and skeletal abnormalities observed at 72 mg/kg/day (highest dose tested) (Pike et al. 1994).
- 5) Mutagenic Effects
  - a) Imidacloprid may be weakly mutagenic. In a battery of 23 laboratory mutagenicity assays, imidacloprid tested negative for mutagenic effects in all but two of the assays. It did test positive for causing changes in chromosomes in human lymphocytes, as well as testing positive for genotoxicity in Chinese hamster ovary cells (Pike et al. 1994).
- 6) Carcinogenic Effects
  - a) Imidacloprid is considered to be of minimal carcinogenic risk, and is thus categorized by EPA as a "Group E" carcinogen (evidence of noncarcinogenicity for humans). There were no carcinogenic effects in a 2-year carcinogenicity study in rats fed up to 1,800 ppm imidacloprid (Anatra-Cordone and Durkin 2005).
- 7) Organ Toxicity
  - a) In short-term feeding studies in rats, there were thyroid lesions associated with very high doses of imidacloprid (Pike et al. 1994).
- 8) Fate in Humans and Animals
  - a) Imidacloprid is quickly and almost completely absorbed from the gastrointestinal tract, and eliminated via urine and feces (70-80% and 20-30%, respectively, of the 96% of the parent compound administered within 48 hours). The most important metabolic steps include the degradation to 6-chloronicotinic acid, a compound that acts on the nervous system as described above. This compound may be conjugated with glycine and eliminated, or reduced to guanidine (USEPA 1995).

## 9) Toxicity to Aquatic Organisms

## a) Fish

## (1) Dose-response

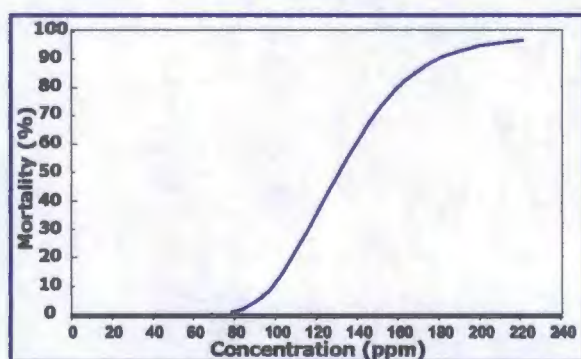
- (a) bluegill (fresh): static 96-hr acute LC<sub>50</sub>, >105 mg a.i./L (Bowman and Bucksath 1990a)
- (b) rainbow trout (fresh), chinook smolts (salt), sheepshead minnow (salt) (Table 2)
- (c) chinook smolts (Figure 1)
- (d) "Using the standard classification scheme proposed by U.S. EPA/EFED (2001), imidacloprid would be classified as practically nontoxic to fish."  
(Anatra-Cordone and Durkin, 2005. Section 4.1.3.1, p 412)

Table 2. Toxicity of imidacloprid to fish (as presented in Anatra-Cordone and Durkin 2005, Appendix 5, except for †, C. Grue, unpublished data 2007)

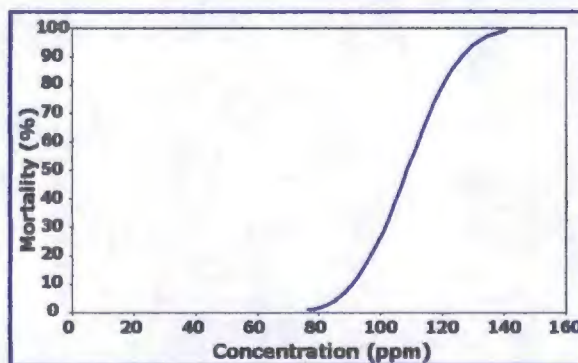
Species	Exposure	Effects	Reference
<b>FRESHWATER Acute Toxicity:</b>			
Rainbow Trout ( <i>Ochorhynchus mykiss</i> ) mean length 5.3 cm, mean weight 1.3 g, 10 per concentration	Static 96-hour acute toxicity study with technical grade NTN 33893 (95.3% a.i.). Nominal concentrations of 0, 50, 89, 158, 281, 500 mg a.i./L, with measured greater than 80% of nominal values	48-hr EC <sub>50</sub> = 85 mg/L, 95% CI = 71 - 113 mg/L 48-hr NOAEC (immobility) = 42 mg/L Mobility was the endpoint of assessment	Young and Hicks 1990 MRID 42055317
Rainbow Trout † ( <i>Ochorhynchus mykiss</i> ) mean weight 0.3 g, 10 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Admire 2F (21.4% a.i.) Nominal concentrations of 0, 15, 22, 32, 46, 66, 96, 139, 202 mg a.i./L	96-hr LC <sub>50</sub> = 170 mg/L, 95% CI = 159 - 181 mg/L 96-hr NOAEC (lethargy) = 22 mg a.i./L (14% at 96 hr)	Grue and Frew unpublished data
Rainbow Trout † ( <i>Ochorhynchus mykiss</i> ) mean weight 23 g, 7 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Admire 2F (21.4% a.i.) Nominal concentrations of 0, 75, 107, 151, 215, 305 mg a.i./L	96-hr LC <sub>50</sub> = 163 mg/L, 95% CI = 148 - 177 mg/L 96-hr NOAEC (lethargy) = < 75 mg a.i./L	Grue and Frew unpublished data
White sturgeon † ( <i>Acipenser transmontanus</i> ) juvenile, mean weight 28 g 5 per concentration	Static 96-hour acute toxicity study with Nuprid 2F (21.4% a.i.) Nominal concentrations of 0, 46, 66, 96, 139, 202, 294 mg a.i./L measured concentrations at: T0 h: 50, 100, and 220 mg a.i./L for nominal of 46, 96 and 202 mg a.i./L; T96 h: 50, 100, and 220 mg a.i./L	96-hr LC <sub>50</sub> = 124 mg/L, 95% CI = 93 - 170 mg/L 96-hr NOAEC (lethargy) = 66 mg a.i./L (Figure 1)	Grue and Frew unpublished data
<b>FRESHWATER Chronic Toxicity:</b>			
Rainbow Trout ( <i>Ochorhynchus mykiss</i> ), newly fertilized eggs <4 hours old, 4 replicates of 35 eggs each per concentration, plus an additional 50 eggs per each of the 4 control replicates (egg viability determination)	98-Day flow-through early life stage test with technical grade NTN 33893 at nominal concentrations of 0, 1.3, 2.5, 5.0, 10 and 20 mg/L equivalent to mean measured concentrations of 0, 1.2, 2.3, 4.9, 9.8 and 19 mg/L	<u>original conclusions:</u> NOAEC = 9.8 mg/L LOAEC = 19 mg/L (statistically significant reduction in length at 36 and 60 days post-hatch, and body weight at 60 days posthatch). No statistically significant biologically important effects on egg viability, hatch, survival or behavioral variables were observed. MATC (maximum acceptable toxicant concentration) = 14 mg/L (geometric mean of NOAEC and LOAEC)	Cohle and Bucksath 1991 MRID 42055320



		<u>1992 re-evaluation:</u> Day 36 growth was most sensitive endpoint. Based on reevaluation of this endpoint: NOAEC = 1.2 mg a.i./L LOAEC = 2.3 mg a.i./L MATC = 1.7 mg a.i./L	Gagliano 1992 MRID 42466501
<b>SALTWATER Acute Toxicity:</b>			
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), young adult, mean length 29 mm, mean weight 0.77 g, 10 per concentration	Static 96-hour acute toxicity test of technical grade NTN 33893 (96.2% a.i.). Control, solvent control, 22.4, 35.2, 58.2, 105 and 195 mg/L mean measured concentrations	96-hour $LC_{50}$ = 161 mg a.i./L, 95% CI = 105 - infinity, NOAEC = 58.2 mg a.i./L on the basis of mortality and signs (lethargy, dark coloration) at higher concentrations.	Ward 1990a MRID 42055318
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), 4-day old, 10 per replicate, 4 replicates per concentration 24-h static renewal	Static 96-hour acute toxicity test of Imida EAG2F (21.4% a.i.) Nominal concentrations of 0, 10, 20, 40, 80, 160 mg a.i./L, mean measured concentrations to verify serial dilutions: 10, 78, and 150 mg a.i./L	96-hr $LC_{50}$ = 61 mg/L, 95% CI = 50-70 mg/L 96-hr NOAEC (lethargy) = 40 mg a.i./L	Frew, Grue and Curran, 2007 unpublished data
Sheepshead Minnow ( <i>Cyprinodon variegatus</i> ), fertilized eggs, 15 per replicate, 4 replicates per concentration ≥ 80% hatch	32-day early life stage toxicity test (USEPA OPPTS 850.1400) of Imida E AG 2F (21.4% a.i.) Nominal concentrations of 0, 0.625, 1.25, 2.5, 5, and 10 mg a.i./L mean measured concentrations to verify serial dilutions: 0.59, 2.3, 9.5 mg a.i./L.	No adverse effects on survival or growth at any concentration tested. NOAEC = 10 mg a.i./L	Curran, Frew and Grue 2008, unpublished report, Nautilus Environmental
Chinook Salmon † ( <i>Ochorhynchus tshawtsha</i> ) mean weight 7 g, 10 per replicate 3 replicates per concentration	Static 96-hour acute toxicity study with Imida 2F (21.4% a.i.) Nominal concentrations of 0, 46, 66, 96, 139, 202, 294 mg a.i./L	96-hr $LC_{50}$ = 109 mg/L (figure 2), 95% CI = 102 - 118 mg/L 96-hr NOAEC (lethargy) = 66 mg a.i./L (Figure 2)	Grue and Frew unpublished data



**Figure 1** Dose-response curve for White sturgeon juveniles exposed to Nuprid 2F in freshwater for 96 hr.  $LC_{50}$  = 124 mg a.i./L, CI = 93 - 170 mg a.i./L. C. Grue unpublished data



**Figure 2** Dose-response curve for Chinook smolts (7g) exposed to Imida 2F in seawater for 96 hr.  $LC_{50}$  = 109 mg a.i./L, CI = 102 - 118 mg a.i./L. C. Grue, unpublished data



(2) Local (Willapa) Field Tests (Table 3; Patten et al., 2007)

(3) Local (Willapa) Lab Tests (Table 4; Patten et al., 2008)

Saddleback gunnel collected in Willapa Bay and maintained in aquaria for 5 days prior to testing. 5 fish per replicate, 3 replicates per concentration. Fish exposed to imidacloprid in estuarine water (56 – 64° F) in 1 L jars.

Table 3. Effects of carbaryl (Sevin) and imidacloprid (Imida) overspray on fish in tide pools.		
Treatment	% survival at 48 hr after treatment	
	staghorn sculpin	threespine stickleback
Sevin 80SP (8 lb a.i./ac)	11.3 <i>b</i>	64.0 <i>b</i>
Imida 2F (0.5 lb a.i./ac)	100.0	100.0
untreated check	100.0	100.0
* means followed by the same letter are not significantly different (Duncans Multiple Range; P=0.05).		

Table 4. Effects of imidacloprid concentration and exposure time on survival of saddleback gunnel ( <i>Pholis ornata</i> ).				
Concentration (ppm)	% Survival			
	4 hr	24 hr	48	96 hr
0	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>	100.0 <i>n.s.</i>
10	100.0	100.0	100.0	100.0
100	100.0	100.0	100.0	93.3
* means followed by the same letter are not significantly different (LSD; P=0.05). <i>n.s.</i> , not significant				

## b) Relevant Aquatic Invertebrates (Freshwater Insects not included)

## (1) Dose Response Parameter (Table 5).

From Anatra-Cordone and Durkin, 2005: "Amphipod crustaceans such as *Hyaella azteca*, the saltwater Mysid, *Mysidopsis bahia*, and the fresh water insect midge, *Chironomus tentans*, are the most sensitive species. In freshwater, the water flea, *Daphnia magna*, was the least sensitive species, while in saltwater, the eastern oyster as least sensitive. Acute toxicity values range from a 96-hour NOAEC of 0.000035 mg/L for *H. azteca* (England and Bucksath 1991), to a 96-hour NOAEC of 145 mg/L for eastern oyster (Wheat and Ward 1991). On the basis of longer-term studies designed to assess reproduction, growth and survival, *M. bahia* was the most sensitive species, with an NOAEC value of 0.000163 mg a.i. imidacloprid/L for growth and reproductive success (Ward 1991), and *D. magna* was the most tolerant species with a 21-day NOAEC for immobility of 1.8 mg/L (Young and Blake 1990)."

Table 5. Toxicity of imidacloprid to relevant aquatic invertebrates (mostly as presented in Anatra-Cordone and Durkin, 2005; Appendix 6).			
Species	Exposure	Effects	Reference
<b>FRESHWATER Acute Toxicity:</b>			
Water flea ( <i>Daphnia magna</i> ), 2 flasks per concentration with 10 each	Static 48-hour acute toxicity study with NTN 33893 (95.9% a.i.) at nominal concentrations up to 125 mg/L with actual mean concentrations of 0, 15, 25, 42, 71 and 113 mg/L	96-hour LC50: 211 mg a.i./L (158 - 281 mg a.i./L). 96-hour NOAEC: 50 mg a.i./L 89 mg/L and higher: apathy, irregular swimming behavior, lying on side/back, staggering 281 mg/L and higher: mortality	Grau 1988a MRID 42055316 Ward 1990a MRID 42055318
<i>Hyaella azteca</i> (amphipod crustacean), 2-3 mm juveniles, 2 replicates per concentration, 10 per replicate	Static acute toxicity test with NTN 33893 at measured concentrations of control, 0.00035, 0.00097, 0.0035, 0.010, 0.034, 0.100, 0.340, 1.000 and 3.100 mg/L	96-hr LC50: 0.526 mg/L, 95% CI = 0.194 - 1.263 mg/L 96-hr EC50 (immobilization): 0.055 mg/L, 95% CI = 0.034 - 0.093 mg/L 96-hr NOAEC (immobilization and abnormal effects, such as lethargy or surfacing) = 0.00035 mg/L	England and Bucksath 1991 MRID 42256303



<i>Hyalella azteca</i> (amphipod crustacean), 14 - 21 days old, 2 replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33823 metabolite at mean measured concentrations of 0, 5.6, 11.0, 22.1, 43.8 and 86.8 mg/L	96-hour LC50: 51.8 mg a.i./L, 95% CI = 44.0 - 60.9 mg a.i./L, 96-hour EC50 (immobilization): 29.0 mg a.i./L, 95% CI = 24.7 - 34.0 mg a.i./L 96-hour NOAEC (mortality): 22.1 mg a.i./L	Rooney and Bowers 1996 MRID 43946601
<i>Hyalella azteca</i> (amphipod crustacean), 7 - 21 days old, 2 replicates per concentration, 10 organisms per replicate	96-hour static acute toxicity of NTN 33519 urea metabolite at nominal (measured) concentrations of 0, 6.25 (5.81), 12.5 (11.80), 25 (23.46), 50 (46.80), and 100 (94.83) mg a.i./L	96-hour LC50: > 94.83 mg a.i./L, 96-hour EC50 (immobilization): > 94.83 mg a.i./L, 96-hour NOAEC: 94.83 mg a.i./L	Dobbs and Frank 1996a MRID 43946603
<b>FRESHWATER Chronic Toxicity:</b>			
Water flea ( <i>Daphnia magna</i> ), 4 replicate jars per concentration, 6 1 <sup>st</sup> instar daphnids per jar	Chronic static renewal toxicity study of technical grade NTN 33893. Control, solvent control, 0.46, 0.86, 1.8, 3.6, and 7.3 mg/L	21-day EC50 (immobilization): > 7.3 mg/L MATC = 2.5 mg/L (1.8 - 3.6 mg/L) NOAEC = 1.8 mg/L LOAEC = 3.6 mg/L 3.6 and 7.3 mg/L: Significantly reduced adult daphnid length in comparison with pooled controls 7.3 mg/L: Significantly reduced survival; significantly reduced mean young/adult reproduction days in comparison with pooled controls. No effects on time to first brood at any concentration.	Young and Blake 1990 MRID 42055321
<b>SALTWATER Acute Toxicity:</b>			
<i>Artemia</i> sp., and Mosquito ( <i>Aedes taeniorhynchus</i> ) 3 trials, 4 replicates per concentration, 10 animals each species per replicate	Static 48-hr acute toxicity test. Technical grade imidacloprid (>95% purity)	<u>Artemia:</u> 48-hr LC50 = 361.23 mg/L, 95% CI = 307.83 - 498.09 mg/L <u>Mosquito:</u> 48-hr LC50 = 0.13 mg/L, 95% CI = 0.010 - 0.016 mg/L Note: increasing salinity increased sensitivity to imidacloprid	Song et al 1997; Song and Brown 1998
Mysid ( <i>Mysidopsis bahia</i> ), < 24 hours old, 10 per concentration.	96-hr flow-through acute toxicity tests of technical grade NTN 33893 (96.2% a.i.). Mean measured concentrations: 1 <sup>st</sup> test: control, solvent control, 0.032, 0.0584, 0.0937, 0.146 and 0.249 mg a.i./L 2 <sup>nd</sup> test: control, solvent control, 0.00842, 0.0133, 0.0229, 0.0372 and 0.0634 mg a.i./L	<u>First test:</u> 96-hr LC50 = 0.0377 mg a.i./L, 95% CI = 0.0267 - 0.0464 mg a.i./L, NOAEC not determined. <u>Second test:</u> 96-hr LC50 = 0.0341 mg a.i./L, 95% CI = 0.0229 - 0.0372 mg a.i./L, NOAEC = 0.0133 mg a.i./L on the basis of mortality and loss of equilibrium at higher doses.	Ward 1990b MRID 42055319
Mysid ( <i>Mysidopsis bahia</i> ), < 24 hours old, 2 replicates per concentration, 10 per replicate	96-Hr flow-through acute toxicity test, NTN 33893 240 FS Formulation, control, solvent control, 18 (21), 29 (31), 49 (56), 82 (78), 136 (125) and 227 (219) ug a.i./L nominal (measured) concentrations	96-hr LC50 = 0.036 mg a.i./L, 95% CI = 0.031 - 0.042 mg a.i./L NOAEC (mortality) = 0.021 mg a.i./L	Lintott 1992 MRID 42528301

Eastern Oyster ( <i>Crassostrea virginica</i> ), 20 per concentration	96-hr flow-through test of effect on shell growth. Technical grade NTN 33893 (95.8% and 96.2% a.i. for 2 <sup>nd</sup> and 1 <sup>st</sup> tests, respectively) 1 <sup>st</sup> test: control, solvent control, 2.93, 5.14, 8.19, 14.2, and 23.3 mg a.i./L, measured 2 <sup>nd</sup> test: control, 145.0 mg a.i./L, measured	<u>First test:</u> 100% survival; No effects on new shell growth <u>Second test:</u> 100% survival; new shell growth of exposed was 22% less than controls. This was statistically significant. 96-hr NOAEC: 145 mg/L	Wheat and Ward 1991 MRID 42256305
<b>SALTWATER Chronic Toxicity:</b>			
Midge ( <i>Chironomus tentans</i> ), second instar, 2 replicates per concentration, 10 chironomids per replicate	Static renewal 96-hr toxicity test with technical grade NTN 33893 (95.0 % a.i.) control, solvent control, measured concentrations of 0.00067, 0.00124, 0.00339, 0.0102, 0.0345, 0.100, and 0.329 mg a.i./L	10-day LC50: 0.00317 mg/L, 95% CI = 0.00124 - 0.0102 mg/L 10-day survival NOAEC: 0.00124 mg/L 10-day growth NOAEC: 0.00067 mg/L (basis = dry weight of survivors)	Gagliano 1991 MRID 42256304
Mysid ( <i>Mysidopsis bahia</i> ), <24- hrs old, 4 replicates per concentration, 15 mysids per replicate cup	Flow-through chronic toxicity tests with technical grade NTN 33893 (96.2% a.i.) <u>First test:</u> control, solvent control, 560, 1290, 2850, 5080 and 10100 ng a.i./L mean measured <u>Second test:</u> control, solvent control, 36.8, 78.4, 163, 326 and 643 ng a.i./L nominal	<u>First Test:</u> <u>1290 ng/L and higher:</u> Significantly reduced number of offspring per female reproductive day <u>5080 ng/L and higher:</u> significantly reduced growth of 1 <sup>st</sup> generation mysids as total length and dry weight <u>10,100 ng/L:</u> Statistically increased mortality in comparison with pooled controls for first generation. No effects on mortality in 2 <sup>nd</sup> generation <u>MATC (reproductive success):</u> 849 ng/L (560 - 1290 ng/L) <u>MATC (growth):</u> 3806 ng/L (2850-5080 ng/L.) <u>Second Test:</u> No effects on number of offspring per female reproductive day. <u>326 and 643 ng/L:</u> Significantly reduced growth of 1 <sup>st</sup> generation as total length and dry weight in comparison with pooled controls <u>643 ng/L:</u> Statistically increased mortality in comparison with pooled controls for 1 <sup>st</sup> generation. No effects on mortality in 2 <sup>nd</sup> generation. <u>MATC (reproductive success):</u> > 643 ng/L <u>MATC (growth):</u> 230 ng/L (163 - 3260 ng/L) <b>No real explanation for discrepancy between 1<sup>st</sup> and 2<sup>nd</sup> tests with regard to growth.</b>	Ward, 1991 MRID 42055322

(2) Local (Willapa) Tests  
(Patten, unpublished data)

i) Diploid oyster larvae  
(a) Survival (Table 5)

All tests featured diploid Pacific oyster larvae from  
Taylor Shellfish within 2 weeks of test. No of  
individuals per replicate and type of arena as

Table 5. Effects of imidacloprid on survival of diploid  
Pacific oyster larvae following 24 hr exposure in 3 arenas.

Arena	Sample Size	Concentration (ppm)	% Survival *
test-tube	15 - 20	0	67.2 n.s.
		1	69.7
		5	47.1
		10	30.7
		20	41.6



specified. 3 replicates per concentration. Tests in water bath at 79 – 80°F for 24 hr. Oysters identified as live or dead based on swimming activity.

Percent survival was not significantly different from plain estuarine water at less than 50 ppm imidacloprid. (Patten unpublished data, 2008)

(b) Survival set, growth (Table 6)

As above, except 4 replicates per concentration; 3 oyster shells per 1 L glass jar. Survival measured after 24 hr exposure and shells transferred to growout bags in Willapa Bay, 6 inches above the tidal substrate, at -1.0 tide height. Number of set oysters and diameter measured after 158 days growout.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

ii) Set, growth of triploid oyster larvae (Table 7)

As above, except triploid Pacific oyster larvae obtained from Taylor Shellfish within 2 weeks of testing, 4 shells per replicate / jar, diameter measured after 172 days in growout bags after 24 hr exposure to imidacloprid.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

iii) Growth of diploid Pacific juvenile oysters (Table 8)

As above, except 5 small juvenile ( $\times$  surface area = 8.5 mm<sup>2</sup>) diploid Pacific oysters per shell, 3 shells per replicate, 3 replicates per concentration, exposed to imidacloprid in fresh estuarine water for 96 hr, then transferred to growout bags for 158 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

iv) Growth of diploid juvenile oysters (Table 9)

As above, except initial juvenile diploid Pacific oyster length was 7.8 mm, 6 oysters per replicate, 3 replicates per treatment, growout for 273 days.

Impact was not significantly different from estuarine water at any concentration or variable (Patten unpublished data, 2008)

250 ml cups	30 – 40	0	15.7 <i>b</i>
		1	10.0 <i>b</i>
		10	18.0 <i>b</i>
		100	0 <i>a</i>
1 L jars	10 – 25	0	48.0 <i>n.s.</i>
		1	28.0
		10	69.0
		20	23.0
1 L jars	30 – 70	0	38.0 <i>b</i>
		5	6.0 <i>b</i>
		50	0 <i>a</i>
		500	0 <i>a</i>

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 6. Effects of imidacloprid on survival, set, and development (diameter) of diploid Pacific oyster larvae after 24 hr exposure.

Sample Size	Concentration (ppm)	% Survival*	No. Set	Diameter (mm)
100 – 150	0	54.5 <i>n.s.</i>	9.3 <i>n.s.</i>	7.8 <i>n.s.</i>
	10	42.0	15.8	8.8
	100	33.0	14.8	8.7
	1000	42.7	18.0	8.6

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 7. Effects of imidacloprid on set and development (diameter) of triploid Pacific oyster larvae following 96 hr exposure.

Sample Size	Concentration (ppm)	No. Set	Diameter (mm)
14 – 150	0	2.4 <i>n.s.</i>	21.9 <i>n.s.</i>
	5	1.3	26.3
	50	1.1	28.1

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 8. Effects of 96 hr exposure to imidacloprid on development of diploid juvenile oysters after 158 days growout.

Concentration (ppm)	Surface Area (mm <sup>2</sup> )
0	8639 <i>n.s.</i>
10	10071
100	9306
1000	7797

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 9. Effects of imidacloprid at 48 and 96 hr exposures on length of juvenile (7.8 mm length) oysters after 273 days growout.

Concentration (ppm)	Length (mm)	
	48	96
0	54 <i>n.s.</i>	48 <i>n.s.</i>
10	53	42
100	37	46
1000	59	39

\* means followed by the same letter are not significantly different (LSD; P=0.05).



v) Growth of juvenile Kumomoto oysters  
(Table 10)

As above, except 5 small juvenile ( $\bar{x}$  diameter = 18 mm<sup>2</sup>)

Kumomoto oysters from Taylor Shellfish per replicate, 3 replicates per concentration, exposed to imidacloprid in fresh estuarine water for 48 or 96 hr, then transferred to growout bags for 92 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

Table 10. Effects of imidacloprid on development (diameter) of juvenile Kumomoto oysters after 24 or 96 hr exposure and 92 days growout.

Concentration (ppm)	Diameter (mm <sup>2</sup> )	
	24 hr	96 hr
0	28.2 <i>n.s.</i>	27.9 <i>n.s.</i>
10	23.4	26.3
100	25.5	27.3

\* means followed by the same letter are not significantly different (LSD; P=0.05).

vi) Manila clams

(a) Preliminary tests by size  
(Figure 3)

Water temperatures for 3 – 6 mm clams, 67° F, others, 48 – 49° F. Survival rates were > 50% for all size classes at imidacloprid concentrations < 1000 ppm (Patten, unpublished data, 2007)

(b) Small clams, (Table 11)

Methods as above for 2008 lab tests, except ~120 small ( $\bar{x}$  diameter = 4.75 mm) Manila clams per replicate / 1 L jar, 5 replicates per concentration. Clams were triple rinsed after treatment then placed on sieved sand. Mortality assessed as not burrowing in sand after 24 hr. Live clams placed in 1 mm mesh growout bags for 30 days, then transferred to 2 mm mesh bags for 46 days.

Impact was not significantly different from untreated estuarine water at any concentration or variable (Patten unpublished data, 2008)

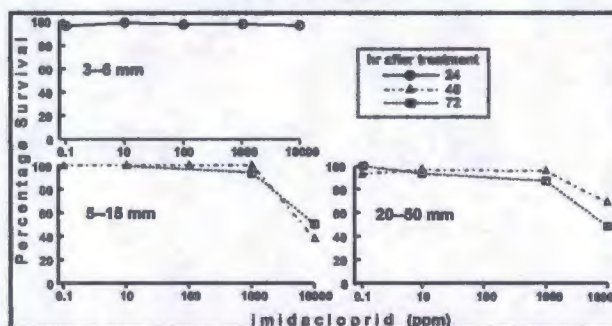


Figure 3 Effects of imidacloprid (Admire 4.6F) on Manila clams of different size classes.

Table 11. Effects of imidacloprid on survival of juvenile Manila clams at exposure intervals and development (diameter) after 76 days growout.

Exposure Interval	Concentration (ppm)	% Survival	Diameter (mm)
48	0	91.7 <i>n.s.</i>	6.0 <i>n.s.</i>
	1	94.5	6.4
	10	90.8	6.9
	100	87.8	5.4
96	0	93.3 <i>n.s.</i>	6.8 <i>n.s.</i>
	1	92.5	7.7
	10	90.2	7.0
	100	91.1	5.9

\* means followed by the same letter are not significantly different (LSD; P=0.05).

vii) Dungeness crab megalopae

(a) Preliminary 2008 trials.

Collected as megalopae using light trap on June 16, 2008, but most metamorphosed to first post-larval instar during exposure to imidacloprid 7 days later. Single individual per replicate, 3 replicates per concentration, 3 exposure intervals per concentration. No mortality at any treatment combination of 0, 10, 100 ppm imidacloprid and 4, 24, 48, and 96 hr exposure intervals.

viii) Juvenile Dungeness Crab

(a) Initial 2007 trials

Mortality was very low in juvenile crab (carapace width < 3") exposed to 0.5 lb a.i./ac imidacloprid in the field (Table 12; Patten unpublished data), but larger crab showed substantial tetanus shock in large scale field trials (see below).

Table 12. Two tests of carbaryl (Sevin 80SP) and imidacloprid (Imida 2F) overspray on juvenile Dungeness crab in tide pools.

Treatment	Days After Treatment	% Mortality*
Sevin 80SP (8 lb	14	70 <i>b</i>
Imida 2F (0.5 lb a.i./ac)	14	0.208333333
untreated check	14	0
Imida 2F (5.0 lb a.i./ac)	21	90 <i>a</i>
untreated check	21	86 <i>a</i>

\* means followed by the same letter are not significantly different (Duncans Multiple Range; P=0.05).



## (b) 2009 trials

Crab were collected as megalopae over three nights in late May and maintained in aerated seawater until testing on May 27. 10 megalopae were placed as a replicate in a 10 ml container containing each of 4 imidacloprid concentrations (0.5, 1, 5, and 10 ppm). Four replicates were exposed to each concentration for 4 hr, after which 5 megalopae per rep were removed rinsed in seawater, and placed in individual 1 L aerated jars. Remaining megalopae were exposed for another 14 hr, and then similarly rinsed and placed in jars. Megalopae were observed for tetanus and mortality at 35 and 131 hr after initial treatment.

Although large percentage of the test populations were in shock at 35 hr after exposure, especially at the higher rate and longer exposure interval, survival was equally high or even greater (Table 13).

## ix) Benthic Infauna (Figure 3; Booth unpublished data, 2007).

Absolute abundance of non-target invertebrates was significantly lower in plots treated with imidacloprid (Admire 1.6F; 0.4 lb a.i./ac) compared to plots treated with carbaryl (Sevin 80SP; 1 lb a.i./ac) or left untreated.

Neither Species Richness nor Simpson's Diversity differed significantly among treatment plots at both short and long post-treatment intervals.

Table 13. Effects of imidacloprid at to induce tetanus shock (measured at 35 hr post treatment) and on survival (at 131 hr post treatment) of crab megalopae.

Exposure Interval (hr)	Concentration (ppm)	% in Shock	% Survival
4	0.5	45	85
	1	55	95
	5	75	80
	10	95	65
18	0.5	100	85
	1	90	85
	5	100	80
	10	100	100

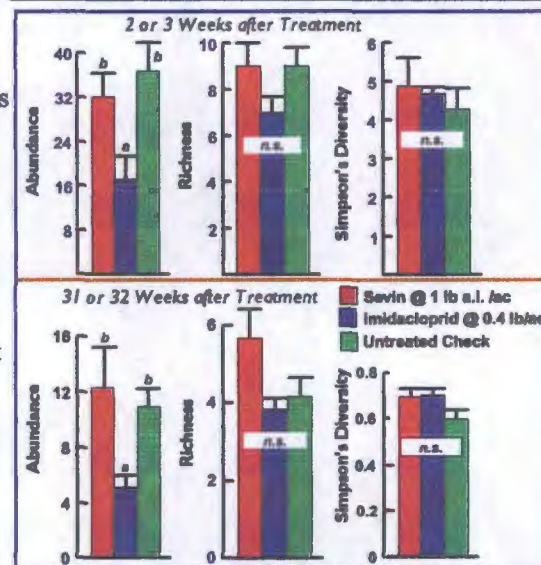


Figure 4 Affects of carbaryl (Sevin 80S) and imidacloprid (Admire 4.6F) on non-target benthic invertebrates at short and long post-treatment intervals.

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#### D) Residue Data

##### 1) Food

- a) In general: an examination of the USDA PDP (Pesticide Data Program) database for FY2004 and FY2005 showed that only about 25% of food samples had detectable imidacloprid residues. Considering that the acute dietary risk assessment scenario assumed that all imidacloprid commodity residues were at tolerance levels and 100% of all crops were treated, the actual acute dietary exposure would be significantly lower than assessed for the Registration Eligibility Decision (Cutchin 2007).
- b) For fish taken for recreation or subsistence consumption under this proposed EUP and associated program: significant exposures to imidacloprid are unlikely given the limited acreage requested and in light of the rapid dissipation of residues following bed treatment (Felsot and Ruppert 2002).
- c) For shellfish: because most beds will be treated with a planted crop of seed which take multiple years of development prior to harvest, the likelihood of any imidacloprid residues remaining unmetabolized is extremely low, especially in light of its  $K_{ow}$ , as explained in Section F. (Petition for Temporary Tolerance) below.
- d) For oysters: using the fugacity based FISH model and appropriate assumptions, estimates of residues in fish (and hypothetically oysters) ranged on a whole body basis from 0.814  $\mu\text{g/kg}$  to 21.1  $\mu\text{g/kg}$  (the assumed body tissue density was 1  $\text{kg/L}$ ). A detailed explanation for the derivation of these concentrations, as well as exposure estimates, are presented in Section F. (Petition for Temporary Tolerance) below.

##### 2) Worker Safety

- a) Exposure estimates for aerial applicators to forest canopy has been calculated at 0.005  $\text{mg/kg/day}$  (Anatra-Cordone, M. and P. Durkin. 2005. Imidacloprid - Human Health and Ecological Risk Assessment – Final Report. Prepared for USDA, Forest Service, Forest Health Protection, GSA Contract No. 10F-0082K, USDA Forest Service BPA: WO-01-3187-0150, USDA Purchase Order No.: 43-1387-4-3131, Task No. 24. Submitted by Syracuse Environmental Research Associates, Inc., 5100 Highbridge St., 42C, Fayetteville, New York 13066-0950)
- b) The re-entry interval (REI) to commercial oyster and clam beds will likely be the same as the labeled REI for other imidacloprid products (e.g., *Admire*, *Guacho*) of 12 hours. The 12 hour restriction has limited relevance, as shellfish workers generally have no need to enter sprayed plots for several days, if not weeks, following application. Shellfish beds sprayed at low tides will also be submerged within 12 hours by the intervening high tides, substantially diluting imidacloprid concentrations in water and on substrate.



**E) Effectiveness Data****1) Small plot trials, 2006 – 2008**

Imidacloprid (Admire 1.6F, Bayer Corp.; Imida 2F, Etigra) has been tested for efficacy against burrowing shrimp since 2006 in several small plot (e.g., 3m<sup>2</sup>, 10m<sup>2</sup>, 10×20m, or 3×20m) trials, as Washington State EUP acreage limit is 0.1 ac per year. Imidacloprid was sometimes applied along with other with compounds (e.g., flowable sulfur, pyrethrins, and pyrethroids), but was most often compared to carbaryl applied at a lower than standard rate (e.g., 3 vs 8 lb a.i./ac) and an untreated check. In initial (2006) broadcast trials, imidacloprid was effective at a range of rates and at a long post treatment interval (Table 14).

Table 14. Affects of carbaryl (Sevin 80WP), 5 rates of imidacloprid (Admire 1.6F) and an untreated check on # burrows/m<sup>2</sup> ( $\bar{x} \pm SE$ ) at 45 and 255 days after treatment (DAT), 2006.

Pesticide	Rate (lb a.i./ac)	Burrow Density*	
		45 DAT*	255 DAT
Sevin	3	16.0 $\pm$ 5.5 a,b	17.3 $\pm$ 3.8 a
Admire	0.05	29.7 $\pm$ 9.4 b	38.0 $\pm$ 6.0 b
	1	15.7 $\pm$ 7.1 a,b	18.0 $\pm$ 9.1 a
	2	1.7 $\pm$ 0.9 a	2.0 $\pm$ 1.0 a
	3	1.0 $\pm$ 0 a	0 a
Untreated	4	0 b	0
	0	73.7 $\pm$ 4.9 c	69.7 $\pm$ 6.9 c

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Our research also included the potential of subsurface injection technologies. In 2004 – 2005, we assessed nozzle and spikewheel injection of non-imidacloprid compounds from semi-amphibious vehicles at low tide. In 2006, a 6' wide apparatus holding 4 spikewheels was mounted on a pontoon raft which was pushed over plots with a boat. Imidacloprid was tested multiple times at various rates and locations using the underwater spikewheel technology. Usually, efficacy of imidacloprid was greater (post treatment burrow density was lower) at higher rates, but the response was not always linear. At a test area near Nahcotta, where substrates were primarily sandy, burrow densities were substantially, if not significantly, higher at rates less than 0.2 lb a.i./ac. This was especially true at longer post application intervals (e.g., 42 or 50 days after treatment) (Table 15, Trials 1, 2). Efficacy was not always greater in plots treated with imidacloprid at rates greater than 0.2 lb a.i./ac (Table 15, Trial 2: 2<sup>nd</sup> and 3<sup>rd</sup> post application interval; Trial 5). Burrow density was also significantly lower in plots treated with 2.0 lb a.i./ac imidacloprid than in plots treated with 3.0 lb a.i./ac carbaryl (Table 15, Trial 1).

Table 15. Affects of carbaryl (Sevin 80SP) and imidacloprid (Admire 4.6F), injected subsurface using underwater spikewheels, on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 5 trials and up to 3 post application intervals (PAI, days after treatment (DAT)) in a sandy substrate at Nahcotta, 2006.

Trial	Treatment	Rate (lb)	Burrow Density*		
			1 <sup>st</sup> PAI†	2 <sup>nd</sup> PAI‡	3 <sup>rd</sup> PAI§
1	Sevin	3	14.7 $\pm$ 3.1 b,c	28.6 $\pm$ 2.9 b	16.4 $\pm$ 1.0 b
	Admire	0.05	23.2 $\pm$ 8.1 c	43.6 $\pm$ 2.9 b	NA
		0.1	5.7 $\pm$ 2.5 a,b	33.1 $\pm$ 2.7 a	NA
		0.2	0.25 $\pm$ 0.2 a	18.2 $\pm$ 1.9 a	13.6 $\pm$ 1.0 a
	Untreated	0	81.0 $\pm$ 2.1 d	91.7 $\pm$ 1.5 c	NA
2	Admire	0.124	23.3 $\pm$ 11.8 a	47.3 $\pm$ 1.6 b	32.4 $\pm$ 1.5 b
		0.25	0.7 $\pm$ 1.2 a	24.9 $\pm$ 3.6 a	17.9 $\pm$ 2.1 a
		0.5	0	22.0 $\pm$ 4.3 a	16.2 $\pm$ 1.9 a
	Untreated	0	62.0 $\pm$ 9.5 b	91.7 $\pm$ 1.5 c	NA
3	Admire	0.2	0.2 $\pm$ 0.2 a	0.7 $\pm$ 0.4 a	NA
	Untreated	0	81.0 $\pm$ 2.1 b	95.3 $\pm$ 3.1 b	NA
4	Admire	0.1	12.2 $\pm$ 2.7 b	NA	NA
		0.2	2.4 $\pm$ 0.7 a	NA	NA
	Untreated	0	72.4 $\pm$ 3.8 c	NA	NA
5	Admire	0.2	6.5 $\pm$ 1.6 a	NA	NA
	Untreated	0	105.4 $\pm$ 4.7 b	NA	NA

\* means followed by the same letter are not significantly different (LSD or t-test; P=0.05).

† Trial 1, 14 DAT; Trial 2, 6 DAT; Trial 3, 10 DAT; Trial 4, 14 DAT.

‡ Trial 1, 42 DAT; Trial 2, 50 DAT; Trial 3, 21 DAT; Trial 4, 21 DAT.

§ Trial 1, 249 DAT; Trial 2, 258 DAT.



Results of a trial conducted on sandy/silty substrates were confounded somewhat by heavy growths of eel grass (primarily invasive *Zostera japonica*, but also *Z. marina*), which slowed tidal drainage, left standing water on the bed, and obscured burrow counts (Table 16).

Another trial, conducted at the Willapa Bay Fish and Wildlife Refuge, featured applications of imidacloprid (Admire 2F; 0.2 lb a.i./ac) on four different types of substrate. Burrows were counted in four 1 m<sup>2</sup> quadrants within and in a single 1 m<sup>2</sup> plot adjacent to each treatment plot. Shrimp burrow density was significantly lower in all treated compared to untreated plots ( $\bar{x} \pm SE$ , 52.2  $\pm$  15.7 burrows/m<sup>2</sup>; LSD, P=0.05), but was significantly higher in a plot of silty hummocks than in plots of other substrate types (Table 17).

In 2007, three broadcast trials continued to demonstrate the fast action and fairly long-lasting efficacy of imidacloprid on burrow density (Table 18).

Table 17. Affects of imidacloprid (Admire 1.6F) at 0.2 lb a.i./ac on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) on different substrate types at 13 days after treatment, 2006.

Treatment	Substrate	Burrow Density*
Admire	Oyster Shell	2.8 $\pm$ 0.6 a
	Silt	3.2 $\pm$ 3.2 a
	Sand / Silt	8.8 $\pm$ 4.3 a
	Silt Hummocks	19.0 $\pm$ 0.6 a

\* means followed by the same letter are not significantly different (LSD; P=0.05).  
Untreated check (52.2  $\pm$  15.7) not included in analysis

Other small plot trials conducted in 2007 and 2008 examined the efficacy of imidacloprid when spikewheel injected by boat or ATV, sediment type, and eelgrass cover on the efficacy of imidacloprid (Table 19). None of the sites featuring application by spikewheel showed outstanding control, whereas burrow density was reduced by  $\geq 95\%$  compared to burrow density in untreated plots when application was by broadcast.

Table 16. Affects of imidacloprid (Admire 1.6F) on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) at 10 days after treatment in sand / silt at Middle Island Sands.

Treatment	Rate (lb a.i./ac)	Burrow Density*
Admire	0.2	4.2 $\pm$ 2.0 a
	0.4	8.1 $\pm$ 1.7 a
Untreated	0	33.5 $\pm$ 2.6 b

\* means followed by the same letter are not significantly different (LSD; P=0.05).

Table 18. Affects of imidacloprid (Imida 2.F) on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 3 trials and at 2 post application intervals (PAI, days after treatment (DAT)) at Nahcotta, 2007.

Trial	Treatment	Rate (lb a.i./ac)	Burrow Density*	
			1 <sup>st</sup> PAI †	2 <sup>nd</sup> PAI ‡
1	Imida	0.5	0	0
		0.25	0.2 $\pm$ 0.1 a	1.8 $\pm$ 0.9 b
		0.125	2.9 $\pm$ 1.1 a	18.3 $\pm$ 4.5 b
	Untreated	0	119.5 $\pm$ 2.4	71.7 $\pm$ 2.4 c
2	Imida	0.5	0	1.3 $\pm$ 0.7 a
		0.25	6.3 $\pm$ 3.1 b	15.0 $\pm$ 4.7
		0	26.1 $\pm$ 4.8	71.7 $\pm$ 2.4
3	Imida	0.5	7.5 $\pm$ 1.6 a	5.8 $\pm$ 2.5 a
		0.25	16.2 $\pm$ 2.3	48.9 $\pm$ 6.2 b
		0	85.6 $\pm$ 3.9	94.7 $\pm$ 5.2 c

\* means followed by the same letter are not significantly different (LSD; P=0.05).

† Trial 1, 7 DAT; Trial 2, 25 DAT; Trial 3, 2 DAT

‡ Trial 1, 99 DAT; Trial 2, 45 DAT; Trial 3, 12 DAT

Table 19. Affects of sediment type, application timing, and application method on efficacy of imidacloprid (0.5 lb a.i./ac) against borrowing shrimp (% reduction in burrows in treated compared to untreated plots). Each row represents a separate experiment.

Sediment Type / Timing	Burrow Density in Untreated Plots (#/m <sup>2</sup> )	Percentage burrow reduction		
		Spikewheel on ATV	Spikewheel on Boat	Broadcast
Sand / April	24	16		62
Sand / May	24	72		62
Sand / July	24	83		96
Sand / September	24	25		95
Silt / June	79		0	49
Sand / June	18		0	96
Eelgrass on sand / August	11	48	74	37



Other trials that featured application by spikewheel lacked a comparison with a broadcast application were conducted on beds with a thin eelgrass cover (Table 20). These trials demonstrated moderate to poor reduction in burrow density, with generally lower efficacies when applications were in August.

## 2) Large scale commercial trials, 2008

### a) Methods

#### (1) Applications

Applications were made according to a Federal Use Permit and accompanying experimental label approved by the EPA. Both contained Directions for Use and Restrictions that were similar to those in the 24C label for use of the standard material, Sevin™, on oyster beds (i.e., do not harvest clams or oysters within one year after treatment, proper and visible flagging of beds, a 200-foot buffer zone must be maintained between the treatment area and the nearest shellfish to be harvested when treatment is by aerial spray; a 50 foot buffer zone is required if treatment is by hand spray, during aerial applications, all public access areas within one-quarter (¼) mile and all public boat launches within a one-and-a-half (1½) mile radius of any bed scheduled for treatment shall be posted). The experimental treatments were applied as similarly as possible to those made for the conventional carbaryl-based program and required the collaboration of the commercial applicator, Dan Foster, and the director of the carbaryl program, Dennis Tufts.

Table 20. Affects of application timing on efficacy of imidacloprid applied using spikewheels on ATV(0.5 lb a.i./ac) against burrowing shrimp (% reduction of burrows in treated compared to untreated plots). Each row represents a separate experiment.

Burrow Density in Untreated Plots (#/m <sup>2</sup> )	Percentage burrow reduction	
	July	August
12	83	
13	87	
29		17
11		72
28		0

Imidacloprid was applied aurally using helicopters to 7 commercial shellfish beds on July 2, 2008 in conjunction with applications of the Sevin, which was applied on July 2, 3, or 7 depending on bed location (Figure 5). Experimental beds were proposed by grower collaborators and selected based on degree of shrimp infestation, size, and proximity to untreated areas or beds treated with Sevin. A 20 ac bed located near the mouth of the North River (A90) had been fallow for at 12 years, had a moderate to heavy shrimp infestation and was isolated from other shellfish beds, so provided a good site to study both efficacy and non-target impact to salmonids. A 10 ac bed near the mouth of the Cedar River (A40) was also used as a site to assess both non-target impact and efficacy. A105 was located in between these sites and had the additional advantage of being accessible from shore. Two smaller beds were located in the Stoney Point growing area (B242 and B183). Two beds were also located in the Oysterville and Nahcotta growing areas (E148 and E163, respectively) where substrate is sandier than the primarily silty substrate of the northern and eastern areas of Willapa Bay. The original intent to match all beds with a nearby untreated area could not always be met. All beds except A105 were inspected prior to application for burrow density, dominant substrate type, amount and kind of eelgrass cover, and other attributes (Table 21).

Table 21. Attributes of commercial oyster beds treated with imidacloprid in Willapa Bay, 2008.

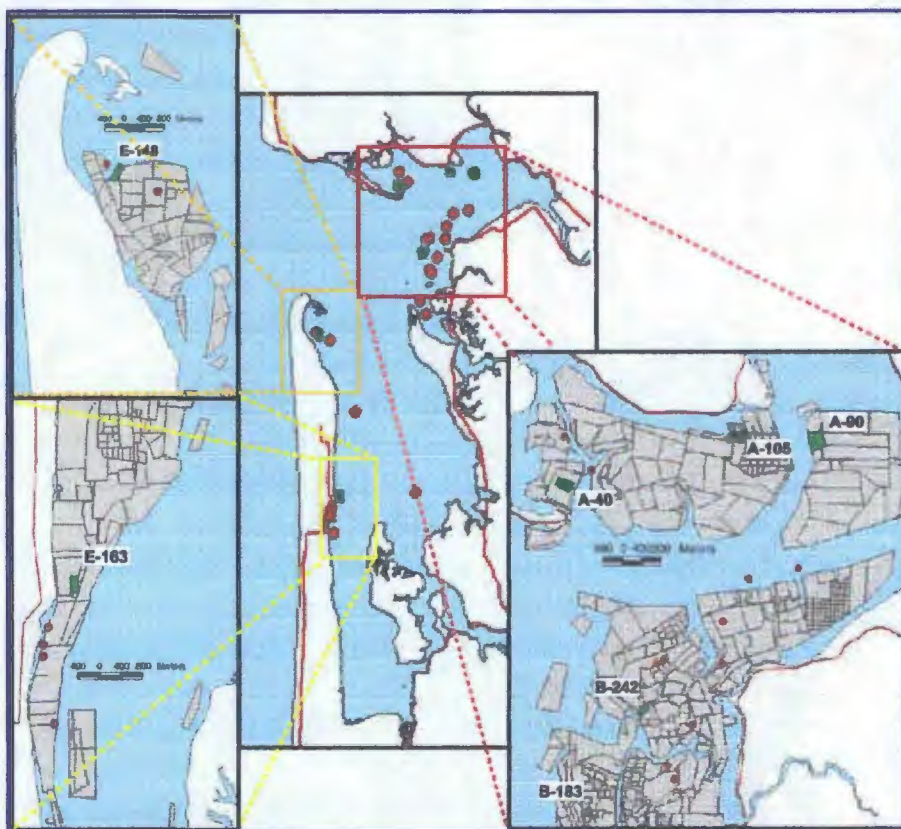
COMPANY	BED NAME	SIZE (ac)	STAGE <sup>a</sup>	LAST TRT <sup>b</sup>	PLANT DATE <sup>c</sup>	ELEV <sup>d</sup>	SP <sup>e</sup>	CUL T <sup>f</sup>	SUB <sup>g</sup>	EEL GRASS <sup>h</sup>	LAT <sup>i</sup>	LONG <sup>j</sup>
Nisbet Oyster	A40	10.0	Cedar R	2006	2008	0.3	G/M	S/H	S/I	heavy †	46.71417	-123.95542
Coast Seafood	A105	10.0	Cedar R	2002	2008	-0.5	M/G	S/H	M/I	heavy †	46.72493	-123.93408
Taylor Shellfish	A90	20.0	Cedar R	pre-95	none	-0.5	G/M	S/H	S/I	patchy †	46.43240	-123.53940
Nisbet Oyster	B242	6.0	Cedar R	2005	2007	1.0	G	S/H	S/I	none †	46.67035	-123.94487
Nisbet Oyster	B183	4.0	Cedar R	2005	2008	1.5	G/M	S/H	M/I	patchy †	46.65178	-123.95228
Northern	E148	10.0	Sheldon	¼-'03, ¼-none	2008	1.0	G/M	S	G/M/S	50% ‡	46.61520	-124.04040
Taylor Shellfish	E163	10.0	Sheldon	never	2008	0.5	G	S/H	S	patchy ‡	46.51505	-124.01963

<sup>a</sup> Helicopter staging area, <sup>b</sup> Year last treated, <sup>c</sup> Year and type of planting, <sup>d</sup> Bed elevation, <sup>e</sup> Species of shrimp (G-ghost, M-mud, G M-ghost dominant, M G-mud dominant), <sup>f</sup> Cultural Type (S-seed, H-harvest, LL-long line, <sup>g</sup> Substrate (M-Mud, S-Sand, I-Silt, G-gravel), <sup>h</sup> approximate density of either (†)native (*Zostera marina*) or ‡ Japanese (*Z. japonica*) density, <sup>i</sup> Latitude (decimal degrees), <sup>j</sup> Longitude (decimal degrees)



Imidacloprid was applied at a rate of 0.5 lb a.i. per ac to 5 of the 7 beds. Due to a mistake, beds in the Oysterville and Nahcotta growing areas were treated at 0.25 lb a.i. per ac. To test the affects of a second half-rate treatment, one half of Bed E163 was treated again 5 days later on July 7.

Two types of ground applications were also tested on the E163 bed: 1) subsurface injection using five Spikewheels™ pulled behind an Argo™ Track ATV and 2) application using 27' spray boom, also mounted on the Argo. Plot sizes were 2 and 5 ac, respectively. Application rate was 0.5 lb a.i. per ac on 1 August.



**Figure 5.** Name, location, size and shape of commercial oyster beds treated with imidacloprid (green) relative to locations of beds treated with carbaryl (red circles indicate points of entry).

## (2) Observations of burrowing shrimp

At all but one site, shrimp burrows were counted both before and at 4 weeks after treatment within a square meter grid placed along transects that criss-crossed the bed diagonally at distance intervals of 5, 10, or 15 paces depending on plot size, to give samples of 30 or more counts per bed. High flood tides sometimes constrained sample size. Counts were averaged within each half transect for statistical analysis.

## (3) Observations of impact to non-target macrofauna

Number of live, dead, or otherwise impaired but visible macrofauna were counted along transects at 5 shellfish beds following the applications. The area at each observation point was roughly 4 m<sup>2</sup> (2 m<sup>2</sup> to the front right and left plus 2 m<sup>2</sup> to the rear right and left). The entire bed could not be covered due to time limitations, but the transects usually crossed the beds diagonally so observations were made at both low and



high ends and at both sides. The number of paces between observation points, and consequent total number of observations, varied according to bed size and duration of the low tide. Three beds, two treated with imidacloprid and one treated with carbaryl, were examined within 1 hr after application. An untreated area near one of the imidacloprid-treated beds that was of similar bed elevation, substrate type, and vegetation cover was also examined as a check. Five beds (2 treated with imidacloprid, 2 treated with carbaryl, and the same untreated bed neighboring the imidacloprid-treated bed) were examined at 24 hrs after treatment.

#### (4) Water samples

Water was sampled for analysis of imidacloprid concentration directly on the bed of three beds and in the adjacent channels of two beds. On-bed samples were taken by grab near the center of the bed, initially when depth of the in-coming tide reached 6" and on subsequent high tides at mid-depth of the water column. In-channel grab samples were taken at both maximum low and high tides at mid-depth of the water column. All samples were held on ice and extracted for imidacloprid analysis within 7 days by Pacific Agricultural Laboratories, Portland, OR.

### b) Results

#### (1) Burrowing shrimp

Burrow density varied substantially at all acrially treated beds, both before and after treatment with imidacloprid (Figure 6). In general, burrow density was significantly lower in beds after treatment with imidacloprid, but levels were not low enough to allow oysters to survive. At the A90 site, burrow density declined significantly from 13.9 at 14 days before treatment to 8.1 at 29 days after treatment (DAT) but was high again 30 days later at 59 DAT. Burrow density also declined in the first 29 DAT, although not significantly, in the nearby untreated area. Due to its drainage patterns and proximity to the North River and a major channel, A90 had a much less regular surface than most other shellfish beds. Burrows on the myriad of small hummocks had been exposed for longer and were much more visible than burrows under

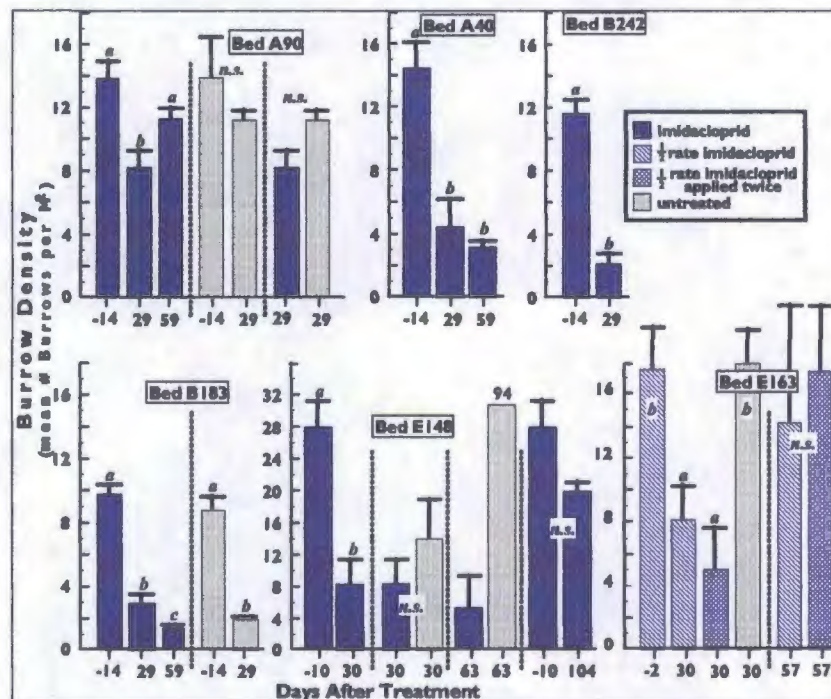


Figure 6. Effects of imidacloprid on burrowing shrimp density at 2, 10, or 14 days before and at 4, 8, or 10 weeks after treatment. Letters "a" and "b" indicate significantly different densities (n.s., not significant).



water. At 58 DAT, mean burrow density on exposed ground was 12.1 compared to 8.9 on ground under  $\frac{1}{2}$  or more inches of water. At A40, number of burrows per  $m^2$  apparently declined to an acceptable level (4.4 burrows/ $m^2$  at 29 DAT and 3.1 burrows/ $m^2$  at 58 DAT), but heavy covers of native eelgrass and algae complicated assessments and could have caused some burrows to be missed. The lack of an adequate untreated control site near A40 also confounded interpretation of results. A similar scenario occurred at B242: burrow density apparently declined significantly and to a potentially acceptable level after treatment with imidacloprid, but heavy vegetation and the lack of a nearby untreated area for comparison confounded the experiment. At B183, burrow density declined in the bed treated with imidacloprid, but also declined in a nearby untreated area in the first 29 DAT. However, the check at B183 was close enough to the treated area that it could have been contaminated by off-site drift. Bed E148, treated with the half rate of imidacloprid, initially showed a similar scenario as that at the A90 site: burrow density was significantly lower at 30 DAT compared to 10 days before treatment, but was still not at an acceptable level for planting. Burrow density was measured as lower at 63 DAT, but not all sections of the bed were examined. A more thorough examination of the bed at 104 DAT gave a higher burrow density.

Shrimp burrow density was also quite variable within beds, especially post-treatment. Some portions of the bed showed moderate burrow density, but other sections were nearly barren. At the first post treatment assessment, comparisons of burrow densities along the transects at some beds showed relatively highly variable post-treatment distributions of shrimp burrows, especially at E163 (Figure 7). At Bed A148, four strips of relatively low burrow density (9.2, 8.3, 1.8, 1.7 per  $m^2$  at a third post-treatment assessment (58 DAT)) were interspersed among stretches of higher burrow density (not counted). Burrow densities at a nearby untreated site were significantly higher (94.4 per  $m^2$ ).

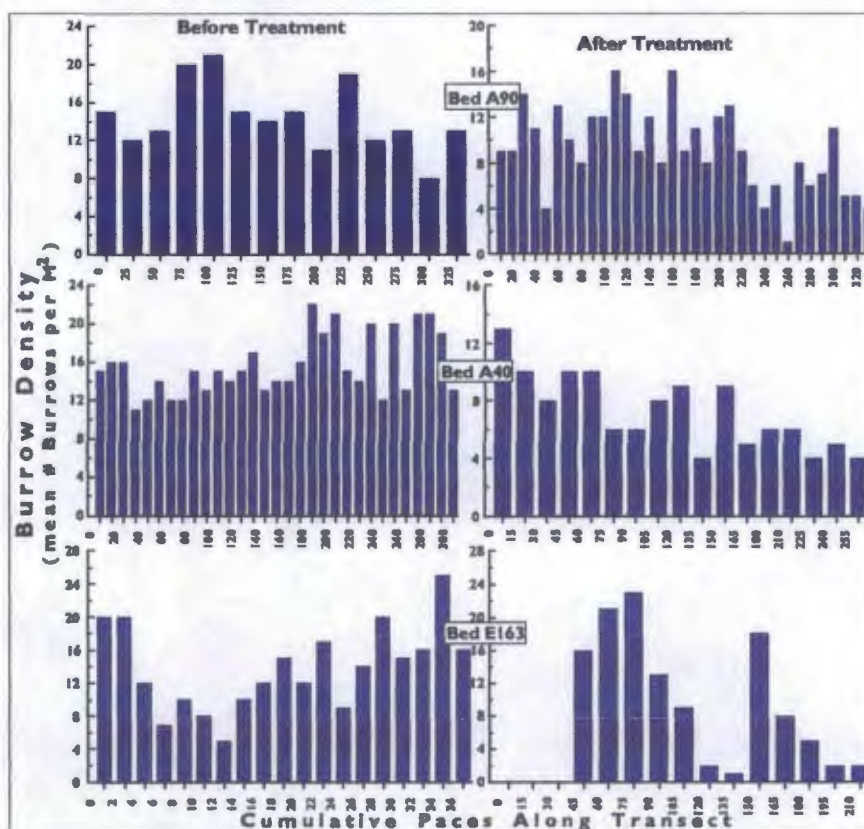


Figure 7 Variation in burrow density along sampling transects at the beds treated with imidacloprid.



Additional observations at E163 showed the patchy distribution of burrow counts to be associated with vegetation, substrate elevation, and related patterns of tidal drainage (Figure 8). At Bed A148, four strips of relatively low burrow density (9.2, 8.3, 1.8, 1.7 per m<sup>2</sup> at a third post-treatment assessment (63 DAT)) were interspersed among stretches of higher burrow density (not counted). The width of these strips (~18 ft) is similar to the width of a spray strip. Burrow densities at a nearby untreated site were significantly higher (94.4 per m<sup>2</sup>).

The ground applications at E163 showed significant reductions in burrow densities in plots treated using either Spikewheels or spray boom compared to both pretreatment levels and densities in an adjacent untreated plot (Figure 9).

(2) Impact to non-target macrofauna, primarily crab

No visibly affected fish were observed. Although a few dead nereid polychaetes were observed at the A90 and the E163 beds, crabs (Dungeness, rock, and hermit) were observed as the most primary animal impacted by imidacloprid (Table 22).

Affected crabs were not dead, but in a state of chronic tetanus shock. They were either entirely exposed or only partially buried and moved very sluggishly when disturbed. Legs and mouthparts were extended and trembled constantly. In comparison, more crab were affected on beds treated with carbaryl and all were dead. Almost all crab were observed in lower areas of the bed or off-bed.

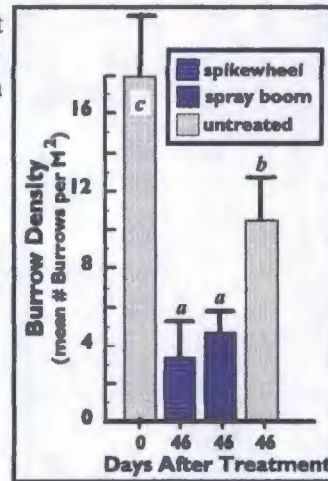


Figure 9 Burrow density in large plots treated with imidacloprid using spikewheels or spray boom.



Figure 8 Distribution of burrow counts among bed attributes at E163 and 63 DAT.

Table 22. Impact of imidacloprid (imid), carbaryl, or no treatment (untreated) on crab, as observed visually at 1 or 24 hours after treatment (HAT).

Bed	Treatment	Treatment Date	HAT	Transects	Paces Between Observations	Observations	Number Crab		
							Normal	Tetanus	Dead
A90	imid	July 2	1	3	1	500	0	0	0
A91	untreated		1	3	1	683	0	0	0
A40	imid	July 2	1	4	5	146	0	0	0
E147	carbaryl	July 7	1	5	1	500	0	0	3
A90	imid	July 2	24	6	5	204	0	15	0
A91	untreated		24	2	5	46	0	0	0
A40	imid	July 2	24	4	5	79	0	6*	0
B183	imid	July 2	24	2	5	65	2	3**	0
E163	imid	July 2	24	7	1	700	0	1	0
A100	carbaryl	July 7	24	4	10	69	3	0	100***
A79	carbaryl	July 7	24	3	20	60	0	0	25****

\* also 10 – 15 lethargic and attenuating crab submerged in drainage channel off lower end of bed.

\*\* also 4 – 8 lethargic and attenuating crab submerged in drainage channel off bed.

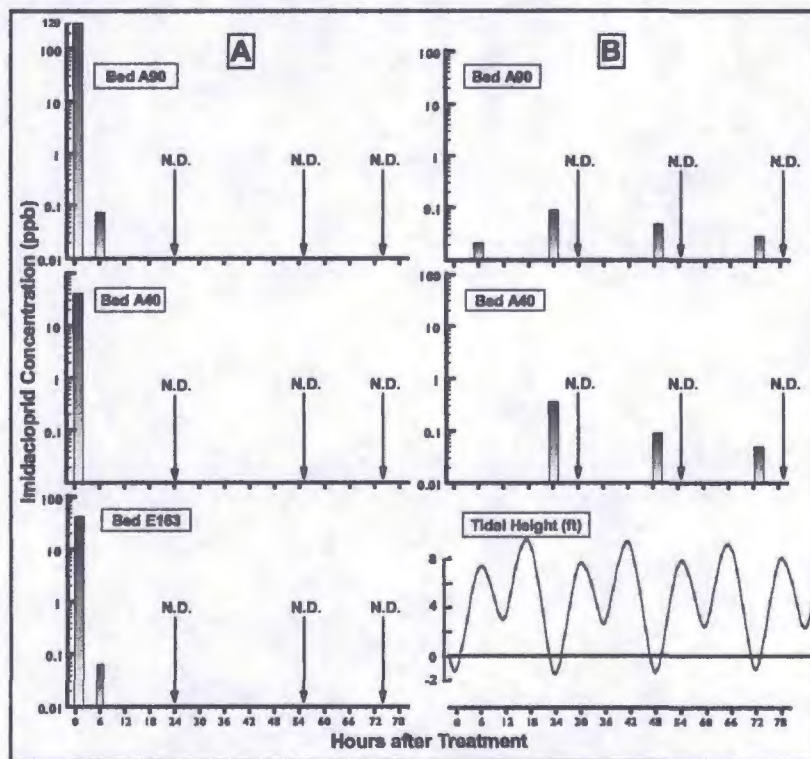
\*\*\* also ~ 100 dead crab in 3×5 m section of drainage channel off lower end of bed.

\*\*\*\* rapidly rising tide prevented off-bed observations.



## (3) Water Samples

Concentrations of imidacloprid sampled over the beds dropped precipitously between 1 and 6 hours after treatment (HAT) and were not detected afterward (Figure 10). Concentrations in the channels adjacent to the beds were recovered from both sample sites at 6 and 24 HAT and at 49 and 74 HAT at one of the sites. These timings were synchronized to the high tides.



**Figure 10.** Concentrations of imidacloprid in water sampled (A immediately over the bed, B) in adjacent channels after applications at ~6 a.m. July 2 and C) tidal fluctuations during the same time at Toke Point near Beds A90 and A40. N.D., not detected (Method Reporting Limit = 0.02 ppb).

## c) Discussion

The general failure of the aerial applications of imidacloprid to suppress burrowing shrimp densities to commercially acceptable levels was due to several factors. The water samples indicated that at least some imidacloprid was transported off-bed during high tide, which likely contributed to generally poor on-bed efficacy against burrowing shrimp relative to carbaryl. Imidacloprid has a lower coefficient of adsorption than carbaryl, so does not bind as tightly to sediments, especially silt, a major component of Willapa Bay tidelands. In addition, most of the beds where efficacy was poor were blanketed with thick vegetation which likely inhibited penetration of imidacloprid. Percent cover of native eelgrass (*Zostera marina*) averaged 67% on Bed A40 and 47% on Bed B183 during pre-treatment assessment while average percent cover of Japanese eelgrass (*Z. japonica*) was 37% on E163. Cover of eelgrass and sea lettuce (*Ulva sp.*) increased during late summer and frequently exceeded 100% in many of the m<sup>2</sup> grids, which greatly confounded measurement of shrimp burrows. At A90, the currents from the North River may have contributed to the already strong tidal currents to wash imidacloprid from the bed before kill. Rising tides approach B183 from both east and west so imidacloprid may not have been washed away as quickly there, resulting in relatively better efficacy. B183 had also been recently dredged so may have retained imidacloprid longer. Impact to non-target macro-fauna was mostly limited to crab and apparently to a smaller portion of the on-bed population compared to carbaryl.

## 3) Small plot trials, 2009

Early season trials have focused on the affects of higher rates and different formulations than the 2F formulation (Nuprid™, NuFarm Inc) at 0.5 lb a.i./ac. Results so far indicate that 5% and 1% granular formulations of imidacloprid (Mallet 0.5G™ and Mantra 1G™, respectively, both NuFarm Inc.) to be highly effective, both alone and when combined with reduced rates of carbaryl (Sevin 80S™, Bayer Corp.) (Tables 23, 24).

Table 23. Affects of imidacloprid formulated as a 5% or 1% granular (Mallet 0.5G, Mantra 1G, respectively) or 2 lb/gal flowable (Nuprid 2F) applied alone or in combination with an 80% wettable powder formulation of carbaryl (Sevin 80WP) on burrowing shrimp (# burrow / m<sup>2</sup>).

Treatment	Rate (lb a.i./ac)	Burrow Density*	
		Pre-treatment †	Post-treatment ‡
Mallet 0.5G	2.0	44.4 <i>n.s.</i>	0.2 <i>a</i>
Mallet 0.5G	1.0	53.2	1.2 <i>a</i>
Mallet 0.5G	0.5	49.6	0.3 <i>a</i>
Nuprid 2F	0.5	56.0	1.2 <i>a</i>
Nuprid 2F	1.0	57.2	0.5 <i>a</i>
Nuprid 2F	2.0	50.8	0 <i>a</i>
Nuprid 2F+Sevin 80WP	0.5 / 2.0	51.2	2.3 <i>a</i>
Nuprid 2F+Sevin 80WP	0.5 / 4.0	50.8	0.3 <i>a</i>
Mantra 1G	1.0	360	0.2 <i>a</i>
Untreated	0	49.2	38.7 <i>b</i>

\* means followed by the same letter are not significantly different (LSD; P=0.05).

† 4 days before treatment, 4/23/09

‡ 8 days post treatment, 5/6/09

Table 24. Affects of formulation and rate of imidacloprid on burrowing shrimp ( $\bar{x} \pm SE$  # burrows/m<sup>2</sup>) in 3 trials and at 10 – 12 days after treatment at Ellen Sands (Trial 1), Sherwood (Trial 2), and WDFW (Trials 3,4), Spring 2009.

Trial	Treatment	Rate (lb a.i./ac)	Burrow Density*	Comments
1	Nuprid 2F	2.0	3.2 $\pm$ 0.8 <i>a</i>	sandy, silt substrate
	Mallet 0.5G	0.50	20.4 $\pm$ 3.2 <i>a</i>	
	Untreated	0	96.4 $\pm$ 2.8 <i>b</i>	
2	Nuprid 2F	2.0	7.6 $\pm$ 0.8 <i>a</i>	sandy, silt substrate light eelgrass know dry time, fast flood tide
	Mallet 0.5G	0.50	4.0 $\pm$ 1.2 <i>a</i>	
	Untreated	0	31.2 $\pm$ 1.2 <i>b</i>	
3	Nuprid 2F	2.0	26.4 $\pm$ 2.4 <i>a</i>	sandy, silt substrate tidal flow 2F in water (2 – 5")
	Mallet 5G	0.50	27.6 $\pm$ 2.0 <i>a</i>	
	Untreated	0	78.8 $\pm$ 2.4 <i>b</i>	
4	Nuprid 2F	2.0	5.2 $\pm$ 1.2 <i>a,b</i>	sandy, silt substrate thick eelgrass cover wet plots / standing water
	Mallet 0.5G	0.50	14.6 $\pm$ 2.0 <i>a</i>	
	Untreated	0	30.8 $\pm$ 1.6 <i>b</i>	
5	Nuprid 2F	2.0	14.2 $\pm$ 1.2 <i>a</i>	sandy, silt substrate thick eelgrass cover wet plots / standing water
	Mallet 0.5G	0.50	27.6 $\pm$ 2.0 <i>a</i>	
	Untreated	0	30.8 $\pm$ 1.6 <i>b</i>	

\* means followed by the same letter are not significantly different (LSD; P=0.05).



**F) Petition for Temporary Tolerance**

An exemption from setting a temporary tolerance is requested in this EUP application based primarily on two lines of argument.

The first argument rests on EPA's own discussions regarding tests for determination of "Accumulation in Laboratory Fish (165-4)". The agency explicitly stated the data requirement was waived for the following reason: "Octanol/water partitioning ( $K_{ow}$ ) data provided by the registrant implies a low potential to bioaccumulate ( $K_{ow}$  for imidacloprid = 3.7 @21°C)" (Parker 2006). These statements imply that the agency review determined that depuration of residues would be very fast and bioconcentration would thus be minimal, especially as concentration following exposure would be widely fluctuating. The rodent metabolism study showed over 90% dissipation of radiolabelled compounds within 24 h suggesting that biological metabolism across species ought to be equally as fast. There is no reason to expect that oysters would not process imidacloprid efficiently as observed in rodents, nor is there any reason to suspect that the bioconcentration or bioaccumulation factor would be significantly different from that predicted for fish.

The second line of argument in favor of a temporary tolerance exemption for this EUP comes from a modeling exercise. A fugacity based model titled FISH Model (version 2, November 2004) is available in the public domain from the Canadian Environmental Modeling Center at Trent University, Peterborough, Ontario, Canada (<http://www.trentu.ca/academic/aminss/envmodel/models/Fish2.html>). The model uses a combination of chemical physicochemical properties and several fish pharmacokinetic parameters to predict whole and lipid fish tissue residue concentrations given a starting point for residues in the water column. Bioaccumulation of chemicals by fish includes both absorption through the gills and food ingestion. The default fish parameters represents fitted parameters from studies with guppies, goldfish, and rainbow trout (Clark et al. 1990). The following analysis makes the assumption that oyster toxicokinetics is similar to that of the default fish model represented in FISH.

The FISH Model was run under two scenarios based on estimated water concentrations and default toxicokinetic assumptions.

First, two imidacloprid water concentrations were used as model input, along with the default fish toxicokinetic parameters: 1) 36 µg/L, the EDWC (estimated drinking water concentration) from EPA's drinking water assessment for imidacloprid yield by the FQPA Index Reservoir Screening Tool (FIRST) (Parker 2006), and 2) 33 µg/L, based on the 0.5 lb a.i./ac, application rate proposed in this EUP application. The application rate was adjusted for depths of water ranging from 1-10 foot. This adjustment is based on Willapa Bay tidal cycles. NOAA data shows a water level change approximating 1.6 ft/hr from low to high tide and back to low tide (<http://tidesonline.noaa.gov/geographic.html>). The highest concentration therefore was estimated to be ~184 µg/L when the water depth was at one foot. The average concentration based on the application rate relative to the algebraic average of all depths during one tidal cycle was 33 µg/L. This concentration was slightly lower than the EWDC from FIRST modeling and thus would hardly change the ultimate exposure perspective.

Second, two default toxicokinetic assumptions in the FISH Model were increased by a factor of 10-fold to increase uptake of imidacloprid by fish and therefore conservatively bias the model output for higher tissue residues. Specifically, the food intake rate was increased from 2% of body weight to 20% of body weight, and the gill resistance factor for the organic phase was reduced from 300 h to 30 h. The other default parameters were not changed. The input water concentrations were the same as in the first scenario (i.e., 36 µg/L and 184 µg/L). Our application of the the FISH Model used imidacloprid concentrations in fish that ranged on a whole body basis from 0.814 µg/kg to 21.1 µg/kg (the assumed body tissue density was 1 kg/L). To estimate exposure, a 0 – 5 year old child was assumed to eat 162 g/day of fish (EPA

Child-Specific Exposure Factors Handbook 2002). This rate was based on the highest mass of fish consumed as recorded in the Columbia River Intertribal Fish Commission study. Exposure estimates, based on a 10-kg child, ranged from 0.0000132 mg/kg/day -- 0.0003418 mg/kg/day.

To characterize the incremental increase in risk that the estimated exposures represented, dietary (food and drinking water) and residential exposure were aggregated. EPA's estimate of aggregate food and drinking water acute exposure was nearly three-fold higher than the chronic exposure value, so it was used in subsequent analyses. For residential exposure, a child with short-term (1-30 day) exposure to a pet treated with imidacloprid was estimated to be higher than other exposure scenarios. EPA did not conduct an intermediate or long-term residential exposure owing to lack of significant hazard in rodent chronic toxicity studies.

The total aggregate exposure was estimated to be 0.15643 mg/kg/day (0.09761 mg/kg/d for dietary/drinking water exposure and 0.05882 mg/kg/day for residential exposure, based on back calculation from an estimated MOE of 170 and a NOEL of 10 mg/kg/day).

The percentage contribution of putative fish tissue exposure was calculated to range from 0.0084% at the low end to 0.2185% at the high end of water residues. Thus, the contribution of fish tissue residues of imidacloprid (and presumably oyster tissues) would not change the overall aggregate risk characterization of imidacloprid.

**In consideration of the EPA's discussions regarding accumulation on imidacloprid accumulation in fish, the results of the FISH model, other observations regarding potential exposure risks (Section D. Residue Data, above), and the isolated location of treated beds (Section G. Proposed Experimental Program, below), we request an exemption from tolerance.**

#### References:

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**G) Proposed Experimental Program****1) Qualifications and Identifications of Participants****a) Researchers***Dr. Kim Patten*

Professor and Extension Specialist

Washington State University

Long Beach Extension Center

Longbeach, WA

**Degrees:**

Undergraduate: 1977

University of California

Davis, CA

Masters: 1980

Iowa State University

Ames, IA

Ph. D.: 1984

Washington State University

Pullman, WA

**Areas of active research:** Dr. Patten is Station Director at the Long Beach Extension Center, where he works in cranberry, shellfish, and invasive weed control.

**Selected Recent Publications:**

Patten, K. and C. O'Casey 2007. Use of Willapa Bay, Washington, by shorebirds and waterfowl after Spartina control efforts. *J. Field Ornithol.* 78(4):395-400

Patten, K. 2006. Review of Clearcast (Imazamox) Aquatic EUP and research results for the western U.S. Proceedings of Aquatic Plant Management Society. August, 2006.

Patten, K. 2006. Parrotfeather milfoil (*Myriophyllum aquaticum*) and water primrose (*Ludwigia hexapetala*) control with herbicides. Proc. of the Western Aquatic Plant Management Society. March, 2006

Patten, K. 2006. Design and evaluate subsurface chemical delivery systems and deep penetrating harrow for management of burrowing shrimp populations. *Shellfish Journal*.

Patten, K. 2005. Burrowing shrimp control. Pacific Coast Shellfish Grower Conference (abstract)

Patten, K. 2005. Watershed mapping of cranberry farms BMPs to reduce surface water pesticides. WSU Extension Conference.

Patten, K. 2005. Invasive Spartina in west coast estuaries. *The Journal of Marine Education* 21:27-31.

Patten, K. 2003. Persistence and non-target impact of imazapyr associated with smooth cordgrass control in an estuary. *Journal of Aquatic Plant Management* 41:1-5.

Hedge, P., L. Kriwoken, and K. Patten. 2003. A review of Spartina management in Washington, USA. *J. Aquatic Plant Management* 41:82-90.

Patten, K. 2003. Eradicating Spartina and restoring affected mudflats using herbicides, new application technologies and supplemental mechanical methods. Abstracts in Invasive Plants in Natural and Managed Systems: 7th International Conference on the Ecology and Management of Alien Plant Invasions. October 2003. Ft. Lauderdale, FL. (abstract).

*Dr. Christian Grue*

Associate Professor, Aquatic &amp; Fishery Sciences

Unit Leader, Washington Cooperative Fish and Wildlife Research Unit

University of Washington

Seattle, WA 98195

**Degrees:**

Undergraduate: 1972

University of California

UC Santa Barbara, CA

Masters: 1977

Northern Arizona University

Flagstaff, AZ

Ph. D.: 1977

Texas A&amp;M University

College Station, WA

**Duties and Research Interests:** Dr. Grue is leader of the Washington Cooperative Fish and Wildlife Research Unit. Dr. Grue's research and that of his graduate students at the University of Washington has focused on the efficacy and non-target effects of chemical and biological pest control within aquatic environments with an emphasis in Washington State and the Pacific Northwest. Recent studies include comparisons in the toxicity among active ingredients, formulated products and tank mixes (end products), effects of *Bti* control of

mosquitoes on aquatic invertebrate communities, and the effects of pesticides in surface waters on the survival and reproduction of salmonids. He teaches a class in fish and wildlife toxicology. Dr. Grue is an active member the Society of Environmental Toxicology and Chemistry and the Wildlife Society and frequently serves on advisory panels dealing with pesticides and other environmental contaminants. He has recently served on FIFRA Science Advisory Panels, the Five-year Review Committee for the USGS's Contaminant Biology Program, and the Editorial Board of the Bulletin of Environmental Contamination and Toxicology, and was recently appointed to the External Advisory Group for the Washington Department of Ecology dealing with the agency's permit for aquatic weed control and eradication.

**Selected Recent Publications:**

- Grue, C.E., S.C. Gardner and P.L. Gibert. 2002. On the significance of pollutant-induced alterations in the behavior of fish and wildlife. Chapter 1 (pages 1-90) in G. Dell'Omo (ed.) Behavioural Ecotoxicology, John Wiley & Sons, Ltd., West Sussex, UK.
- Major, W.W., III, C.E. Grue, SC Gardner and J.M. Grassley. 2003. Concentrations of glyphosate and AMPA in sediment following application of Rodeo® to control smooth cordgrass in Willapa Bay, Washington. Bulletin of Environmental Contamination and Toxicology 71:912- 918.
- Curran, C.A., J.M. Grassley and C.E. Grue. 2004. Toxicity of R-11® surfactant to juvenile rainbow trout: Does size matter? Bulletin of Environmental Contamination and Toxicology 72:401-408.
- Smith B.C., C.A. Curran, K.W. Brown, J.L. Cabarrus, J.B. Gown, J.K. McIntyre, E.E. Moreland, V.L. Wong, J.M. Grassley and C.E. Grue. 2004. Toxicity of four surfactants to juvenile rainbow trout: Implications for over-water use. Bulletin of Environmental Contamination and Toxicology 72:647-654.
- Getsinger, K.D., M.D. Netherland, C.E. Grue and T.J. Koschnick. 2008. Improvements in the use of aquatic herbicides and establishment of future research directions. Journal of Aquatic Plant Management (In press).
- Grue, C.E., C.A. Curran J.L. Cabarrus S.C. Gardner, N. Spang, J.M. Grassley, B.C. Smith, and K.A. King. Active ingredients, formulations and tank mixes: What should be regulated? Integrated Environmental Assessment and Management (In external review).
- Tamayo, M., C.E. Grue and L.L. Conquest. Response of wetland invertebrates to mosquito control. Journal of Applied Ecology (External review completed, submission December 2007).
- King, K.A., W.L. Madden, C.A. Curran, R.A. Battin Jr, C.T. Elfes, S.R. Frame, J. Kim, M.T. McDaniel, V.A. Pelekis, M.R. Sternberg, J.M. Grassley, and C.E. Grue. Brain AChE inhibition in juvenile rainbow trout exposed to pesticide mixtures within urban streams in western Washington: Non-additive effects. Bulletin of Environmental Contamination and Toxicology (Ready for external review).
- Grue, C.E., C.T. Elfes, S. Booth, B.R. Dumbauld, A.S. Felsot, N.C. Overman, J.M. Grassley, and W.W. Major III.. Commentary — Behavioral impairment and increased predation mortality in cutthroat trout exposed to carbaryl: Leaps of faith and pious hopes. Marine Ecology Progress Series (Submission December 2007).

***Dr. Vince Hebert***

Laboratory Research Director,  
Food and Environmental Quality Laboratory  
Washington State University-Tri Cities  
Food and Environmental Quality Lab  
Richland, WA

**Degrees:**

Undergraduate: 1983	Masters: 1987	Ph. D.: 1999
Humboldt State University	University of Nevada	University of Nevada
Arcata, CA	Reno, NV	Reno, NV

**Areas of active research:** 1) developing analytical methods for assessing specific biomarkers useful for monitoring pesticide exposures to sensitive subpopulations in agricultural communities, 2) the development of field air -sampling methods and volatilization chamber



system design for assessing fumigants, pesticides, and semiochemicals useful in codling moth mating disruption, 3) characterizing/isolating bioactive plant volatile emissions from insect herbivory that may prove useful in enhancing conservation biological control in cropping systems, and 4) chemically assessing sublethal concentrations of pesticides in surface waters that can have neurobehavioral effects on salmonids. A principle responsibility is to administer over a state-mandated food and environmental regulatory science facility that conducts studies under federal 40CFR Part 160 Good Laboratory Practices (GLP). This program houses an independent quality assurance unit and GLP Laboratory Coordinator to assure federal compliance.

Selected Recent Publications:

- Hebert VR and Miller GC. Understanding the tropospheric fate of agricultural pesticides, in Reviews of Environmental Contamination and Toxicology, ed. G. Ware, Vol. 181 pp 1-36 (2004).
- Woodrow J, Hebert VR, LeNoir J. "Monitoring Of Agrochemical Residues In Air." in "Handbook of Residue Analytical Methods for Agrochemical Residues" (P. Lee ed., two volume series) John Wiley & Sons. pp. 908-935 (2003).
- Merriman J, Hebert VR Methyl Isothiocyanate Residential Community Air Assessment; South Franklin County, Washington. Bull of Environ Contam and Toxicol. In press (Jan 2007)
- Hebert, VR. Understanding the tropospheric transport and fate of semivolatile pest management chemicals. In: Environmental Fate and Safety Management of Agrochemicals ACS Symposium Book Series 899, ed. JM Clark, pp 70-82 (2005).
- Hebert, VR, Hoonhout C, Miller GC. Reactivity of certain gas-phase organophosphorus insecticides toward hydroxyl radicals at elevated air temperatures. J. Agric. Food. Chem, Vol. 48: (2000): 1922-1928.
- Hebert, VR, E Tomaszewska, J. F. Brunner, V. P. Jones, and M. Doerr. Evaluating the pheromone release rate characteristic of commercial mating disruption devices. In Crop Protection Products for Organic Agriculture. Environmental, Health, and Efficacy Assessment. Felsot, A. S., K. D. Racke (ed.); Am. Chem. Soc., Symposium Series 947, Am. Chem. Soc., Washington, DC. pp. 144-157 (2006).
- Weppner, S, Elgethun K, Lu C, Hebert VR\*, Yost M, Fenske R. The Washington aerial spray drift study: Children's exposure to methamidophos in an agricultural community following fixed-wing aircraft application J. Expos. Anal. Environ. Epidem 16: 387-396 (2006).

*Dr. Alan Felsot*

Professor and Extension Specialist  
Entomology and Environmental Toxicology  
Washington State University-Tri Cities  
Food and Environmental Quality Lab  
Richland, WA

Degrees:

Undergraduate: 1972	Masters: 1974	Ph. D.: 1978
Tulane University	University of Florida	Iowa State University
New Orleans, LA	Gainesville, FL	Ames, IA

Research and Extension Interests: Hazard assessments of transgenic crops, pesticide drift and buffer zone design, reduction of insecticide application rates using new sprayer technologies, enhanced biodegradation of pesticides, remediation of pesticide waste in soil, best management practices for controlling agrochemical movement to surface and ground water, analytical chemistry of pesticide residues in soil, water, and food, pesticide toxicology, regulations, and risk communication. He teaches a graduate course entitled "Applied Environmental Toxicology." He also team teaches the course, "Pesticides: Toxicology and Modes of Action."

Recent Publications:

- Felsot, A. S. 2004. Establishing buffers: Protocols and toxicological benchmarks, Proc. International Conference on Pesticide Application for Drift Management. Oct 27-29, Waikoloa, HI. pp. 199-203.
- Felsot, A. S. 2004. Impact of U.S. court cases on application technology, Proc. International Conference on Pesticide Application for Drift Management. Oct 27-29, 2004, Waikoloa, HI. pp. 53-58.
- Felsot, A. S. 2004. Is the content of disease-reducing phytochemicals influenced by certified organic

crop production practices? Paper no. 21, 228th National Mtg. American Chemical Society (PICOGRAM Issue no. 67, p. 55), Aug 22-26, 2004. Philadelphia, PA.

Ramaprasad, J., M.-Y. Tsai, K. Elgethun, V. R. Hebert, A. Felsot, M. G. Yost, R. A. Fenske. 2004. The Washington aerial spray drift study: assessment of off-target organophosphorus insecticide atmospheric movement by plant surface volatilization. *Atmospheric Environment* 38:5703-5713.

Felsot, A. S., 2004. No-spray buffer zones for the ag/urban interface: derivation using drift modeling and toxicologically relevant benchmarks (26 MB \*.pdf). Paper no. 85, 227th National Mtg. American Chemical Society (PICOGRAM Issue no. 66, p. 68), Mar 28-Apr 1, 2004. Anaheim Calif.

#### b) Consultants

*Dr. Alan Schreiber*

President, Agriculture Development Group, Inc., Pasco Washington  
 Administrator - Washington State Commission on Pesticide Registration  
 Executive Director - Washington Asparagus Commission

##### Degrees:

Undergraduate: 1984	Masters: 1987	Ph. D.: 1991
Northeast Missouri St. Univ.	University of Missouri	University of Missouri
Kirkville, MO	Columbia, MO	Columbia, MO

Research and Extension Interests: For the Ag Development Group, Dr. Schreiber consults on environmental, pesticide, pest management and Food Quality Protection Act issues for grower groups, governmental organizations and agribusiness's and conducts research on more than 30 crops on a 100 acre research farm. For the WSCPR, a state governmental entity dedicated to support of activities related to pesticide registration and pest management, Dr. Schreiber manages a \$0.9 million budget and interacts with all commodity and pest management groups, pest management researcher and extension specialist in Washington. Prior to these positions, Dr. Schreiber was Assistant Professor for the Department of Entomology, Washington State University, and before that, Entomologist for the USEPA/Office of Pesticide Programs/Biological and Economic Analysis Division

##### Honors and Awards:

Outstanding Service Award to U.S. Potato Industry, National Potato Council, 2002  
 Entomological Society of America, Excellence in Extension nominee, 1997  
 WSU Outstanding Extension Scientist, Department of Entomology nominee,  
 1997 Oregon/Washington Asparagus Growers Assn. "Friend of the Industry Award,"  
 1996 Columbia Basin Vegetable Seed Association Outstanding Service Award, 1995

*Dr. Steven Booth*

PSI / WGHOGA  
 120 State St. NE #142  
 Olympia, WA 98501

##### Degrees:

Undergraduate: 1975	Masters: 1982	Ph. D.: 1992
University of Iowa	Western Washington Univeristy	Oregon State University
Iowa City, IA	Bellingham, WA	Corvallis, OR

Research and Extension Interests: As the IPM Coordinator for the Willapa Bay / Grays Harbor Oyster Growers Association, Dr. Booth assists in the development and implementation of a variety of chemical, biological, and mechanical tactics for the control of burrowing shrimp. He has writes grant proposals to fund the IPM program and reports that describe its progress. Prior to his current position, Dr. Booth has developed IPM tactics featuring biorational pesticides, insect parasitic nematodes and fungi, and beneficial insects.

##### Recent Publications:

Booth, S.R., Drummond, F. and E. Groden. 2007 Special considerations for application and evaluation



- of entomopathogens in specific systems: Small fruits. *in* Field Manual of Techniques for the Use and Evaluation of Entomopathogens, 2<sup>nd</sup> Edition. [L. Lacey and H. Kaya, eds., Ch. VII.12. Kluwer Press. pp 583 – 598.
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- Booth, S.R., Tanigoshi, L.K., and Shanks, C., Jr. 2002. Evaluation of entomopathogenic nematodes to manage root weevil larvae in Washington state cranberry, strawberry, and red raspberry. *Env. Entomol.* 31:895-902.
- Booth, S.R., Tanigoshi, L.K., and I. Dewes. 2000. Potential of a dried mycelium formulation of an indigenous strain of *Metarhizium anisopliae* against subterranean pests of cranberry. *Biocontrol Science and Technology* 10:659-668.
- Booth, S.R. and C.H. Shanks. 1998. Potential of a dried rice/mycelium formulation of entomopathogenic fungi to suppress subterranean pests in small fruits. *Biocontrol Science and Technology*. 8:197-206.

**c) Grower Cooperators – members of WGHOGA who own acreage allotments**

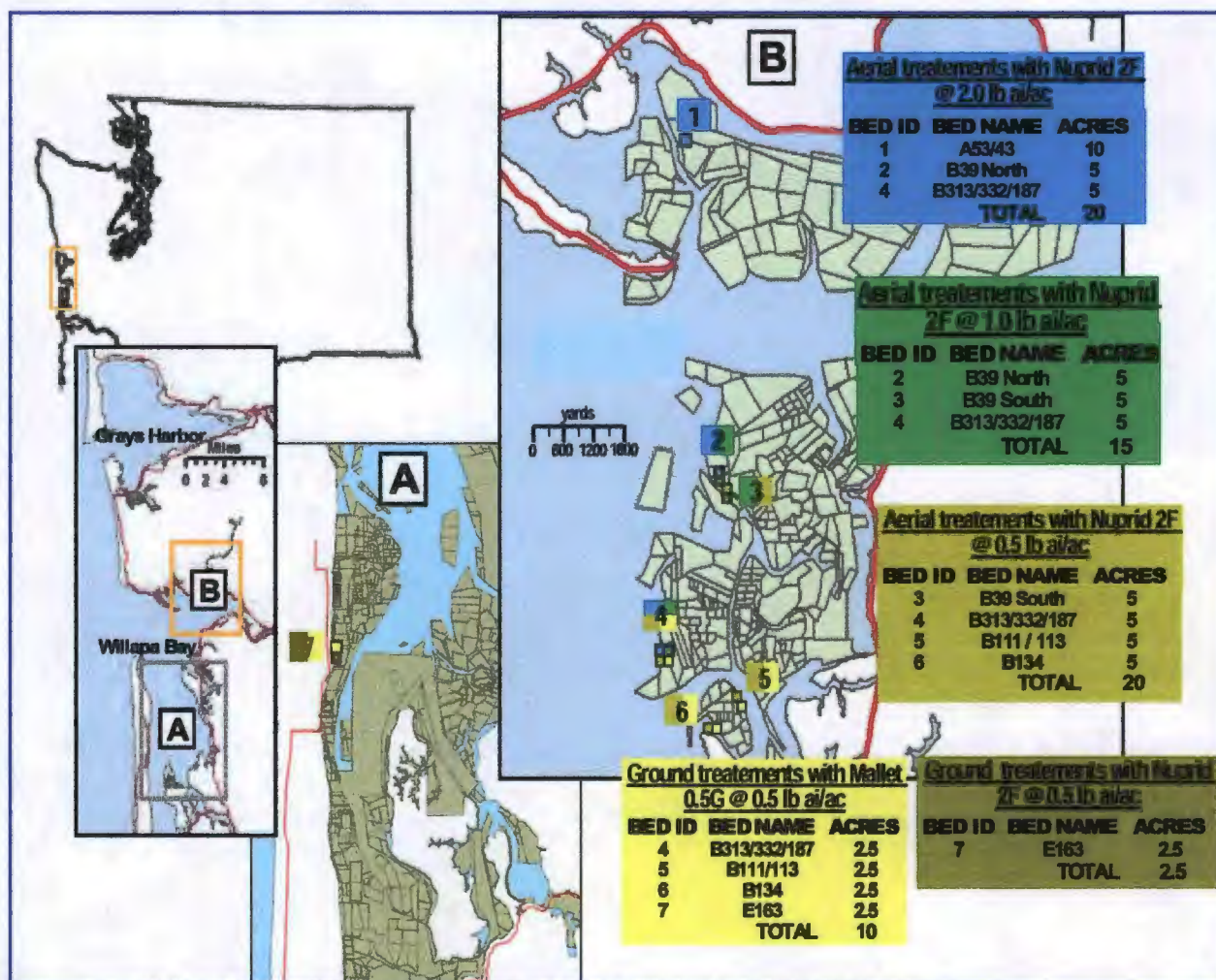
<i>Kristi Ballo</i> Brady's Oysters 3714 Oyster Pl. E. Aberdeen, WA 98520	<i>Nick Jambor</i> Ekone Oyster Co. 29 Holtz Road South Bend, WA 98586	<i>Jerry Swan</i> Grass Creek Oyster Co 1975 Lakemoore Pl SW Olympia, WA 98512
<i>Leonard Bennett</i> R&B Oyster Co P O Box 309 Bay Center, WA 98586	<i>James Kemmer</i> Long Island Oyster PO Box 1054 Long Beach, WA 98631	<i>Bill Taylor / Eric Hall</i> Taylor Shellfish Co., Inc. SE 130 Lynch Road Shelton, WA 98584
<i>Dan Driscoll</i> Oysterville Seafarms P O Box 6 Oysterville, WA 98641	<i>Tim Morris</i> Coast Seafoods Box 166 South Bend, WA 98586	<i>Dennis Tufts</i> Wilson Oyster Co. PO Box 236 Ocean Park, WA 98640
<i>Don Gillies</i> Stony Point Oyster Co. L.L.C. 6931 US Hwy 101 South Bend, WA 98586	<i>Dave Nisbet</i> Nisbet Oyster Co. PO Box 338 Bay Center, WA 98527	<i>Fritz Wiegardt</i> Wiegardt & Sons P O Box 309 Ocean Park, WA 98640
<i>John Heckes</i> Heckes Clam Co P O Box 1657 Ocean Park, WA 98640	<i>Phil Olsen</i> Olsen & Son Oyster Co. PO Box 212 South Bend, WA 98586	<i>Dr. Richard Wilson</i> Bay Center Mariculture P O Box 356 Bay Center, WA 98586
<i>David Hollingsworth</i> Markham Oyster Inc. 20 Old Westport Road. Aberdeen, WA 98520	<i>Brian Sheldon</i> Northern Oyster Company PO Box 1039 Ocean Park, WA 98640	

**2) Locations, acreage to be treated**

All areas to be treated lie within the 4,250 intertidal acreage of Willapa Bay and Grays Harbor (4250 ac (Feldman et al. 2000) and 7,500 ac (<http://graysharbor.fws.gov>), respectively). Most of the 35,000 commercial acreage (BSCC 1992) lie several hundred meters from land and human habitation. A maximum of 67.5 ac intertidal ac will be treated with imidacloprid. However, treatments will feature different combinations of liquid soluble concentrate imidacloprid (Nuprid 2F; NuFarm America, Inc.) applied at three different treatment rates (2.0, 1.0, and 0.5 lb a.i./ac) and 0.5% granular imidacloprid (Mallet 0.5 G; NuFarm, Inc.) applied at 0.5 lb a.i./ac. Six of 7 study sites will be located within Cedar River / Stony Pt/ Palix River growing area, with a 7<sup>th</sup> located in the Nahcotta growing area on the Long



Beach Peninsula (Figure 11). Beds were selected based on density of burrowing shrimp, substrate type, grower cooperation, ease of access, size, proximity to beds targeted for carbaryl application, proximity to untreated areas, and proximity to known salmonid populations. None of the target beds lie within 200 yards of a major channel or oysters that will be harvested within 1 year.



**Figure 11** Name, acreage, and location of shellfish beds to be experimentally treated with imidacloprid in the Nahcotta (A) and Cedar River / Stony Pt / Palix River are in Willapa Bay on July 21, 2009.

### 3) Details of the Proposed Program

All treatment variables cannot be compared at each study bed (e.g., a factorial experimental design) due to both a lack of study sites and a desire to minimize potential impact in this second year, still preliminary large scale trials. Instead, selected treatment variables, primarily imidacloprid formulation (Nuprid 2F (liquid) or Mallet 0.5G (granular)) and rate of liquid imidacloprid (Nuprid 2F), will be compared at paired study plots of different treatment combinations. Five paired comparisons will be conducted at 5 study beds: (2.0 lb a.i./ac vs 1.0 lb a.i./ac of Nuprid 2F, 1.0 lb a.i./ac vs 0.5 lb a.i./ac of Nuprid 2F, and 0.5 lb a.i./ac of Nuprid 2F vs Mallet 0.5G. Four formulation/rate treatment combinations will be compared at one site. Nuprid 2F will be applied aerially using helicopters on July 21 in association with the conventional carbaryl program at 6 of the 7 sites; a spray boom apparatus mounted on an ATV will be employed at the 7<sup>th</sup> site. Mallet 0.5G will be applied at 0.5 lb a.i./ac using conventional ground-based granular dispensers at 4 sites on the same day or day before the aerial applications. Nuprid 2F will be applied at 2.0 lb a.i./ac alone to an isolated 10 ac bed near the Cedar River channel to assess both efficacy between areas of high eelgrass density and bare ground. That same site will be used to assess imidacloprid



impact on salmonids, crabs, and to monitor imidacloprid off-site transport and dissipation in the sediments. All treatment beds are adjacent to areas of similar substrate, vegetation, and burrow density that will remain untreated as checks.

Carbaryl will be applied to other beds in the general vicinity on July 22, none of which will be located closer than 1000 m to beds targeted for imidacloprid treatment. Carbaryl will be applied to beds immediately adjacent to one of the study beds (B39) on July 8, two weeks before the experimental imidacloprid treatments.

or otherwise adequately represent the bed. Percentage cover of eelgrass, algae, shell, and standing water will also be recorded.

Trials and assessments of efficacy will be directed primarily by Dr. Kim Patten, Long Beach Research Unit, Washington State University and Dr. Steven Booth, Pacific Shellfish Institute. For both small plot and commercial scale trials, efficacy will be judged primarily by comparing shrimp burrow counts taken before treatment and at several post treatment intervals (~4 – 8 weeks and, pending results, 11 months after treatment). On commercial beds, the length of the interval before sampling will also depend on when seed is planted. Walking on newly planted seed will substantially damage the crop. Efficacy on each bed will also be eventually and ultimately be judged by yield.

Non-target field impact on the benthic in-fauna will be addressed by the Pacific Shellfish Institute, using protocols which have been approved for Willapa Bay by the Washington State Department of Ecology. Three core-replicates cores will be taken at each of 4 sites near the corners of the study plots treated with imidacloprid at 2.0 lb a.i./ac. Cores will be taken using a PVC clam gun. Similar samples will be taken in nearby untreated beds. Cores will be taken at 2 – 4 weeks prior to treatment and 1 month post treatment. Each core (15 cm deep x 10.2 cm in diameter) will be immediately sieved through 0.5 mm mesh using salt water and stored in a 10% buffered formalin solution for 2 weeks, then stained with rose bengal and re-sieved through 250 um mesh to remove excess detritus and stored in 70% ethanol. Polychaete identification and enumeration will be to species by Dr. Eugene, Ruff Wormworks, Inc., Puyallup, WA. Identification and enumeration of other invertebrates will be conducted by personnel at PSI. Species attributes (type and abundance) of key benthic invertebrates, as well as community descriptors (Abundance, Species Richness, and Simpson Diversity) will be used to compare treatment affects.

Non-target and sub-lethal effects on salmonids (i.e., juvenile chinook and cutthroat trout) will figure heavily in both the federal registration and state permitting of imidacloprid. A biomarker, based on imidacloprid residues in brain tissues, was successfully tested by Dr. Christian Grue, University of Washington, to address these effects. The biomarker showed good correlation between residues, created from precisely controlled exposures of chinook smolt to a range of imidacloprid concentration, to selected physiological functions (gill ATPase activity) or non-function (mortality), and overt behavioral effects (lethargy, erratic swimming, on-bottom gilling). These findings will be validated this year.

An ancillary study continues last years' tests on the utility of existing ELIZA test kits for imidacloprid residues in brain tissues. Last years' results showed high correlation among a range of imidacloprid residue concentrations identified in the brains of cutthroat trout using the ELIZA kit and standard laboratory methods.

We shall also begin preliminary assessments of the impact of the imidacloprid applications on crab populations. These will include observations of crab caged on treated beds, and 24 and 48 hr post bed inspections for dead or crab in tetanus shock.

A preliminary fate & transport study will measure concentrations of imidacloprid in water over the bed, in off-bed channels, and in bed sediment pore water at several post treatment intervals. Most of these samples will occur in conjunction with the salmonid impact studies conducted on and near Bed A43/A53 near the Cedar River channel.

#### 4) Objectives

- a) At the commercial scale, and alongside the conventional carbaryl-based aerial treatment plan, compare the efficacy of imidacloprid against burrowing shrimp to the carbaryl (Sevin 80S; Bayer Corp) standard and untreated checks according to three primary variables:
  - (1) formulation of imidacloprid:
    - i) (Nuprid 2F (liquid))
    - ii) Mallet 0.5G; NuFarm, Inc. (granular)
  - (2) rate of Nuprid 2F
    - i) 2.0 lb a.i./ac
    - ii) 1.0 lb a.i./ac
    - iii) 0.5 lb a.i./ac
  - (3) vegetation type
    - i) thick eelgrass or algal mats
    - ii) moderate densities
    - iii) bare ground
- b) In smaller (<0.1 ac) plots, compare efficacy of the two formulations according to more combinations of these same three variables (formulation, rate, and substrate type) as well as others (bed elevation, application timing, and presence of oyster seed)
- 5) On and near sites of an isolated large aerial imidacloprid treatment (10 ac of Nuprid 2F @ 2.0 lb a.i./ac), assess impact to non-target organisms:
  - (1) salmonids (e.g., juvenile Chinook and cutthroat trout)
  - (2) other fish
  - (3) Dungeness crab
  - (4) benthic infauna
- b) At that same isolated site, and selected sites of granular treatment, make preliminary assessments of imidacloprid off-bed transport in the water column and dissipation in sediment pore water.

#### 6) Explanation and Justification of Quantity

These trials will require 71.25 lb a.i. of imidacloprid to be applied to a total of 67.5 total acres in Willapa Bay (Table 25). The requested acreage is required to complete the studies required for imidacloprid registration and permitting in the second of a multi-year experimental program (see point 6 below). Amounts were derived according to an experimental design that strives for suitable replication but is constrained by limited space, time, and considerations for potential non-target impact. Our most common plot size (5 ac) tend to the low size of most commercial beds ( $\geq 10$  ac)

but are still large enough to include some variation in burrowing shrimp density, substrate, vegetation, bed elevation, and drainage pattern that accompany commercial shellfish beds and impact efficacy. Plots to be

Table 25. Acreage and quantity of imidacloprid proposed for experimental application in Willapa Bay in 2009 according to formulation (Nuprid 2F (liquid) or Mallet 0.5G (granular), and rate.

Material	Rate (lb a.i./ac)	Acreage	lb a.i.
Nuprid 2F	2.0	20.0	40.0
Nuprid 2F	1.0	15.0	15.0
Nuprid 2F	0.5	22.5	11.25
Total Nuprid		57.5	66.25
Mallet 0.5G	0.5	12.0*	6.0
Total Mallet		10.0	5.0
Total imidacloprid		67.5	71.25

\* includes 10 ac commercial trials plus 2 total ac ancillary small plot (<1 ac) trials



treated with the granular formulation are smaller (2.5 ac) as these preliminary trials will feature ground-based application that will be somewhat limited by the weight of the material. The 0.5% material will require 250 lb to treat 2.5 ac at 0.5 lb a.i./ac.

Two additional acres are requested to test the granular material in small (<1 ac) plots.

#### **7) Duration**

We request that a federal experimental use permit for imidacloprid on Washington state shellfish grounds be granted for one year with anticipated renewals for at least the two following years.

We have prioritized and timed studies according to a two year registration and four year permitting process for completion in 2012. The figure shows activities planned primarily for 2008 and, to a lesser degree, 2009 and 2010. The results of studies conducted in 2009 and 2010 will determine what studies will be conducted in 2012. These include the completion of the registration process and a major modification of the current NPDES permit to include imidacloprid, which will be renewed in July 2011. As noted above, the requested acreage will likely change from year to year as well. A more complete and precise timeline for the registration of imidacloprid on Washington state shellfish grounds cannot be constructed at this time. There is little precedent for an aquatic use for this compound, so federal and state requirements have yet to fully specified.

#### **8) Disposition of unused material**

Almost all imidacloprid will be used during experimental application, as the amount of material applied will be precisely measured and applied using calibrated equipment. Unused material will be stored temporarily in an EPA and OSHA compliant pesticide storage unit located at the Washington State University Research and Extension Unit in Long Beach, WA. Material will eventually be disposed through the Washington Department of Agriculture's Pesticide Disposal Program.